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STUDIES ON SURFACE TAINT BUTTER[1]

I. ODOUR PRODUCTION BY *PSEUDOMONAS PUTREFACIENS*[2]

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INTRODUCTION

Pseudomonas putrefaciens has been reported to be a cause of surface taint butter by Derby and Hammer (3), Wolochow (13), and Linneboe (6), of rabbito butter by Loftus-Hills, Scharp and Searle (7) and of putrid butter by Claydon and Hammer (2).

For the recognition of surface taint butter there is not yet available any criterion more precise than odour production. It has been assumed that the odorous substance causing the defect is a protein degradation product (3). The student of the problem is quickly confronted with the difficulty of distinguishing with certainty the many putrefactive and other odours and with the possibility of more than one defect being included in the practical or experimental grading of such defective butter.

Surface taint was first observed in Canadian butter in 1919 by Marker (8) who described and named the defect. The affected butter was a shipment of Alberta butter on the Vancouver market.

The authors have been unable to find an authentic instance in Canada of a similar defect prior to this date and no authentic case of the defect in raw cream butter has been brought to their attention. Surface taint has appeared sporadically in creamery butters of Alberta during each year since 1919. An acceptable explanation for surface taint, therefore, will be required to provide a reason for the sudden, spectacular and continued appearance of the defect in creamery butters made in this province. This requirement should serve as a useful guide in delineating for the experimentalist the practical boundaries of the surface taint problem.

METHODS

The strain of *Ps. putrefaciens* used in these studies was from a culture kindly supplied at the start of the project by Linneboe (6). Throughout the work it was frequently purified.

[1] This series of papers is a joint contribution from the Division of Bacteriology and Dairy Research, Science Service, Dominion Department of Agriculture, Ottawa, and the Department of Dairying, University of Alberta, Edmonton. Part of the data contained therein is taken from a thesis presented by the senior author to the University of Alberta in partial fulfilment of the requirements for the degree of Master of Science. Contribution No. 129 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Dominion Department of Agriculture, Ottawa, Canada.

[2] In this series of papers *Pseudomonas putrefaciens* is used synonymously with *Achromobacter putrefaciens* (Derby and Hammer (3)). The taxonomic relationships leading to the change in name will be discussed in the fourth paper of the series.

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The skim milk was spray skim milk powder reconstituted 10% in water. The plating medium was tryptone-glucose-beef-extract-2% skim milk agar. Hydrogen ion concentrations were measured electrometrically. All butter samples were incubated in an ice chest at 10° C. to 15° C., while cultures and plates were incubated either at this temperature or at room temperature.

In the earlier stages of the work the butter samples were graded by the Dominion Dairy Produce Graders, Edmonton. Later, the grading was done largely by those associated with the studies with frequent check grading by the Dominion Dairy Produce Graders in the interests of uniformity.

The churning cream was pasteurized at a minimum of 81° C. for 10 minutes in gallon lots in an experimental size, stainless steel pasteurizer with hot water as the heating medium.

Churning was accomplished in motor-driven Dazey churns equipped with stainless steel paddles and held in a water bath. Rotating 2-quart sealers had previously been found to permit only indifferent control of temperature and agitation. The resulting butters of about $\frac{1}{4}$ to $\frac{1}{3}$ pound per churning were worked with sterile tongue depressors on wet sterilized boards. At no time was it found possible to reproduce, to the satisfaction of all the butter judges, the thorough incorporation of moisture which is characteristic of Alberta creamery butter. Unless otherwise stated no salt was added to the butter. The study involved a total of about 400 experimental churnings.

ODOUR PRODUCTION IN LIQUID MEDIA

Sterile Skim Milk

When grown for 24 hours or longer at room temperature in test tubes of heat-sterilized skim milk, *Ps. putrefaciens* produced a typical odour which, though slight, was discernible and distinguishable in the confined atmosphere above the surface of the milk. When a few drops of such milk were spread on the fingers and allowed to dry to the point of stickiness, this odour was greatly intensified. In approximately 2500 random isolations of various species of bacteria from creamery well waters and normal and abnormal creamery butters, this odour and this characteristic were unique with *Ps. putrefaciens* and a yellow bacterium to be described in the sixth paper of this series. Until proof is forthcoming that the substance causing this odour and the substance causing the characteristic odour of surface taint butter are identical, it was deemed advisable to distinguish the former by the descriptive term "sweaty feet odour" and retain the term "surface taint" for the butter defect.

There was no intensification of the sweaty feet odour when skim milk cultures of *Ps. putrefaciens*, with or without acidification, were exposed in thin layers in petri dishes or when so exposed in petri dishes smeared with butter, butter-oil, lecithin or glycerol.

When the concentration of skim milk powder in water was 4% or less, the sweaty feet odour could not be detected with certainty after the growth of the organism.

Other Media

Only a very slight sweaty feet odour was noticed when the organism was grown for 9 days at room temperature in the neutralized and autoclaved whey from H_2SO_4 precipitated skim milk. Similar observations were made with rennet whey. On the other hand butter serum of low salt content proved to be a good medium for the production of the odour. A faint but definite sweaty feet odour was produced from nutritive caseinate broth (Difco) and quite distinctly from 5% sodium caseinate in water. It was not produced, at least unequivocally so, from casein (Hammersten) or a commercial sodium caseinate (2%) dispersed either in peptone water or in Kisch's synthetic medium. Thus it would appear that the precursor of the odorous substance follows the casein fraction but it has not yet been otherwise related to casein.

It was not determined with certainty, either in the ordinary way or by aeration or distilling techniques, if the sweaty feet odour was produced in nutrient broth, peptone or tryptone water. A weak sweaty feet odour was observed in cultures in 5% and 10% concentrations of Bacto peptone and Bacto tryptone, and was probably present in 2% peptone water. The presence of 5% glycerol in 2% peptone water intensified the odour slightly but noticeably. In none of these cases, however, was the odour pronounced. The odour was not present with certainty when the organism was grown in aqueous solutions of edestin, egg albumin, lactalbumin (either raw or heat denatured), lactoglobulin (either raw or heat denatured) or peptonized milk. There was no indication that any of the following compounds, when dissolved or suspended in peptone water, was the source material:— β-alanine, alanine, arginine, cysteine, cystine, glutamic acid, leucine, isoleucine, norleucine, lysine, methionine, threonine, tryptophane, tyrosine, valine, glutamine, glucosamine, lactose, glucose, fructose, maltose, sucrose, butyric acid, caproic acid, caprylic acid, capric acid, oleic acid, lactic acid, or lecithin. The organism did not change the odour coming from nutrient broth containing from 2 mg. per cent to 20 mg. per cent indole. When indole was added to skim milk cultures in like concentrations, the indole odour was easily distinguishable from the sweaty feet odour.

pH

There is evidence (4, 15) strongly suggesting that the odorous substance produced in skim milk by *Ps. putrefaciens* is a volatile acid of low molecular weight. If such is the case, it would exist as a salt in alkaline solutions and would be freed as an acid at lower pH's.

When *Ps. putrefaciens* was grown in skim milk at pH's of 7.6 or higher a typically putrid odour was discernible. When such milk was then acidified the sweaty feet odour was apparent, presumably because the substance was released as the free acid.

When skim milk cultures of the organism were aerated in an aeration train, the sweaty feet substance was held in NaOH solutions, while typically putrid odours were recognizable at the exit. On the other hand the sweaty feet substance emerged when passed through H_2SO_4 solutions, while no typically putrid odours were recognized at the outlet. Whenever the sweaty feet odour was discernible, odours suggestive of the goat acids were also distinguished.

Volatile fatty acids are also present in the steam distillate of acidified skim milk cultures of *Ps. putrefaciens* (4, 15). In the above aeration experiments acidification of the residue from the NaOH solutions released both goat acid and sweaty feet odours.

In parallel experiments with skim milk cultures of *B. subtilis* and *Ps. fluorescens* the sweaty feet odour was at no time suggested, while the typically putrid and volatile fatty acid odours were present and easily differentiated organoleptically by those associated with the study. It may be significant that a number of buttermakers were unable to differentiate the three types of odours, *i.e.*, the typically putrid, the volatile fatty acid and the sweaty feet odours. In certain experiments with unworked and melted butters, practical butter men graded as surface taint many samples the odours from which did not suggest either surface taint or sweaty feet to the authors. This points to an explanation for certain obscurities enveloping this problem and to a necessity for care in the grading of experimental surface taint butters.

Since the sweaty feet defect produced in skim milk by *Ps. putrefaciens*, and surface taint in butter caused by the same organism appear to be but a form of rancidity, and since such putrefactive organisms as *Ps. putrefaciens*, *Ps. fluorescens* and *B. subtilis* all produce volatile fatty acids in skim milk, the possibility of rancidity in dairy products arising from a protein or other non-fat source deserves experimental consideration (5, 9, 10).

E_h

Litmus was reduced in skim milk by *Ps. putrefaciens* at room temperature over night. The organism was so strongly reducing that even aeration for 8 days failed to reoxidize litmus milk cultures. At no time was the sweaty feet odour observed prior to the reduction of the litmus and at no time was the odour apparent in the tubes while the litmus was oxidized. Thus the odour disappeared from reduced cultures which were oxidized by heating and then cooling or by the addition of H_2O_2 or quinhydrone. The odour returned in each case, however, after the culture was spread on the fingers for some time.

Odour production was inhibited in skim milk by (1) 0.00034 N hydroquinone, (2) 0.002 N $CuSO_4$ and (3) 0.01 N $FeSO_4$ but not by (1) 0.005 N KCN, (2) 0.002% diphenylamine or (3) 0.01 N Na_2SO_3.

Diacetyl in a concentration of 100 p.p.m., but not of 1000 p.p.m. in litmus milk, permitted the growth of the organism. No sweaty feet odour was observed in the cultures containing 100 or 10 p.p.m. while 1 p.p.m. seemed to have no effect on odour production. The presence of added acetyl-methyl-carbinol or 2,3-butylene glycol in varying concentrations had no observable effect on the course of the defect, except that the odour of the latter compound in concentrations of 1000 p.p.m. or higher was sufficient to have masked the sweaty feet odour had it been present.

While it was noticed that the sweaty feet odour is more easily detectable in some than in other atmospheres, it was not possible to relate the difference to any factor.

It may be that the sweaty feet compound is produced by *Ps. putrefaciens* in a reduced state and in a concentration insufficient for organoleptical detection; that the compound becomes partially oxidized and

detectably odorous at E_h values of the general level of those of milk in O_2 equilibrium with the air, while further oxidation at more positive E_h levels again results in an odorless state; and that these reactions are reversible. Further work is necessary to justify conclusions in this regard. Such a theory, however, is tempting in view of the well-known relation of surface taint to freshly-cut surfaces of butter.

Heat Treatment

On a number of occasions milks were drawn aseptically from both Holstein and Jersey cows not known to have a history of udder inflammation. The whole milks, skim milks and gravity-separated creams were aseptically dispensed into sterile test tubes and subjected to various heat treatments, as indicated below, before inoculation and incubation.

In no case was the sweaty feet odour detected after incubation of the raw milks. In the majority of the tubes heated to 62.8° C. for 10 minutes there was no suggestion of the sweaty feet odour. In a few tubes, if the odour was present at all, it was so slight as to render positive recognition impossible. In the tubes heated to 62.8° C. for 30 minutes the sweaty feet odour was definitely present but only slightly so. In the tubes heated to 71.1° C. or 82.2° C. for 10 minutes the odour was present as characteristically as when the organism is grown in skim milk previously autoclaved for 10 or 20 minutes. The observations on the control and heat-treated skim milks and creams did not vary from the above.

ODOUR PRODUCTION IN BUTTER

Heat Treatment

Raw and pasteurized creams were inoculated from broth cultures of *Ps. putrefaciens* and incubated for 12 to 18 hours prior to churning. The resulting butters were firmed overnight in the ice chest in open sterilized ointment jars. By means of threads, cubes with all freshly-cut surfaces were then prepared and incubated as above in closed sterilized ointment jars for periods up to 16 days.

In no case did butter made from raw cream grade surface taint, while in no case did the pasteurized-cream butters fail to grade surface taint. Butter did not grade surface taint which was churned from pasteurized, inoculated, incubated and repasteurized cream. Surface taint butter did not result from the churning of inoculated whipping cream commercially pasteurized, presumably at low temperature. It is interesting to note that Brown (1) reported that a defect of New South Wales butter which he described as "disagreeable aroma" was not inducible in raw-cream butter.

Butter churned from inoculated cream which had been pasteurized by the vacreator process did not develop surface taint. In parallel churnings with vacreated cream repasteurized in the laboratory at 81° C. for 10 minutes before inoculation, the defect appeared on the resulting butters in the usual manner. It would seem that during vacreation the cream is not held at high temperature long enough to permit those chemical changes which are a prerequisite to surface taint development.

Oxidants, Antioxidants, etc.

Surface taint production in butter was delayed but not prevented by various concentrations of an aqueous extract of Avenex No. 3. It was

delayed and sometimes even prevented in the presence of 0.05% and 0.1% hydroquinone. Isolations of *Ps. putrefaciens* from poured plates showed that this effect was not caused by a bactericidal action of the hydroquinone.

The addition to inoculated cream of from 57 to 104 p.p.m. of Cu (as $CuSO_4$) gave butters which developed a "brown-sugar" odour rather than typical surface taint. After 20 days storage at 10° C. to 15° C. the odour was one of strong tallowiness. The addition of from 12 to 35 p.p.m. of Cu had a delaying action on the appearance of surface taint. Plate counts showed no definite influence of Cu in limiting bacterial numbers. Isolations from the plates showed that *Ps. putrefaciens* was the principle member of the flora of all the inoculated samples, regardless of Cu content.

Freshly-cut cubes of surface taint butter did not show the defect when held for $2\frac{1}{2}$ days at 10° C. to 15° C. in a rarefied atmosphere freed of O_2 by alkaline pyrogallol. Under similar conditions, a freshly-streaked agar slope of the organism failed to show growth. Upon removal from the O_2-free air, surface taint appeared in 12 hours on the butter, and growth on the slant became evident. Although these results permit of two interpretations, it seems probable that the rapid appearance of surface taint on freshly-cut surfaces, an outstanding characteristic of this defect, involves a chemical rather than a bacteriological change.

Diacetyl in concentrations of 0.18, 1.8 and 18 p.p.m. was added to inoculated cream prior to churning. It is seen from the grading results as presented in Table 1 that the presence of diacetyl not only delayed the appearance of surface taint but also markedly lessened the severity of

TABLE 1.—THE EFFECT OF ADDING DIACETYL TO CREAM ON THE DEVELOPMENT OF SURFACE TAINT IN THE BUTTER

		Grade							
		Control	Inoculated 12 hours prior to churning				Inoculated immediately prior to churning		
Diacetyl in P.P.M.		0	0	18	1.8	0.18	18	1.8	0.18
Butter Storage Period	20 hours	C	ST(P)	D	D ST(S)	ST(S)	D	C	C
	28 hours	C	ST(P)	D ST(S)	D ST(S)	ST(S)	D	C	ST(S)
	2 days	C	ST(P)	D ST(S)	ST(M)	ST(M)	D	ST(S)	ST(S)
	3 days	C	ST(P)	D ST(S)	ST(M)	ST(M)	D	ST(S)	ST(S)
	4 days	C	ST(P)	ST(M)	ST(M)	ST(M)	D	ST(S)	ST(M)
	9 days	C	R	R	R	R	D	U	U
	3 weeks	U	U	U	U	U	U	U	F

ST—Surface Taint; (P)—Pronounced; (M)—Moderate; (S)—Slight; D—Diacetyl; C—Clean; U—Unclean; R—Rancid; F—Mouldy.

the defect. The effect of the diacetyl was more pronounced when the cream was inoculated immediately prior to churning than when inoculation preceded churning by 12 hours. It may be, therefore, that the inhibiting action of diacetyl varies inversely with the degree of contamination with this organism. The course of the defect was not altered in the presence of added acetyl-methyl-carbinol or 2,3-butylene glycol in varying concentrations.

Claydon and Hammer (2) did not observe putridness in experimental butter in the presence of 5% or more of starter. The results with diacetyl cited above provide at least a partial explanation for Claydon and Hammer's observations as well as for the rarity of surface taint in the poorer grades of Western Canadian butters.

Sodium Chloride

Salting at the rate of 1% and 2% apparently delayed the production of surface taint. At 3%, surface taint was completely inhibited only if accompanied by thorough working. Salt and diacetyl appeared to act independently of each other.

Working

The rate of appearance and severity of the defect tended to vary inversely with the degree of working of the experimental butters.

Acidity

Cream adjusted to pH 4.85 with sterile lactic acid yielded normal butter when either the cream or the wash water was inoculated. Cream adjusted to pH 4.75 yielded normal butters whether the inoculation took place immediately before or 12 hours before churning. Surface taint resulted when inoculated-cream butter was washed with water adjusted to pH 4.9 with sterile lactic acid or 0.25 M phosphate buffer.

Sterile lactic acid, when worked into inoculated-cream butters at the time of manufacture, had no effect on the course of the defect if the pH of the butter serum was 5.6 or above. The defect did not appear if the

TABLE 2.—THE EFFECT OF THE PH OF THE BUTTER SERUM ON THE DEVELOPMENT OF SURFACE TAINT IN THE BUTTER

Inoculation of cream	Lactic acid added	Percentage acid added	Serum pH	Grade on 6th day	Grade on 12th day
Uninoculated		0	6.65	Clean	Clean
Inoculated	During manufacture	0.042	5.28	Clean	Clean
		0.062	4.85	Clean	Clean
		0.082	4.35	Clean	Clean
	6 days later	0	6.45	S.T.	S.T.
		0.042	5.50	S.T.	S.T.
		0.062	5.50	S.T.	S.T.
		0.082	4.45	S.T.	S.T.

adjusted pH of the butter serum was 5.3 or below. It did not cause the disappearance of the defect when worked into experimentally-produced surface taint butter, even if the acidities were brought below pH 5.3. It was observed that the odour from these latter butters was remarkably similar to that of butters adulterated with steam-distillate residue from skim milk cultures of *Ps. putrefaciens*. The data in Table 2 illustrate the effect of working sterile lactic acid into butters churned from variously-treated portions of a sample of cream.

These findings confirm those of Claydon and Hammer (2) that a pH of 4.5 in the churning cream prevented the development of putridness in butter caused by *Ps. putrefaciens*.

The Inoculum

Surface taint butter was regularly produced whether the inoculum was a milk culture, a broth culture or a water suspension from an agar slant growth of *Ps. putrefaciens*.

Steam-distillate Residue

Crude steam-distillate residue from skim milk cultures of *Ps. putrefaciens* (15) was added in varying amounts to sweet pasteurized cream prior to churning. In most concentrations in the resulting butters the odours differed distinguishably from typical surface taint. There was agreement among those associated with the study that in one concentration the odour was very nearly like that of typical surface taint. There was disagreement, however, as to whether the two odours were identical. A fraction of volatile acids, containing a high percentage of isovaleric acid and recovered from milk cultures of *Ps. putrefaciens* (15), when added to churning cream, did not impart a typical surface taint odour to the resulting butter.

Butter Fat Emulsions

Surface taint butter was reconstituted at 82.2° C. in skim milk to give a cream of approximately 32% fat. When cooled, the reconstituted cream was churned and the resulting butter incubated as usual. Surface taint did not reappear on this butter.

By means of a small pharmaceutical hand homogenizer, clarified butterfat from normal butter was reconstituted in 3 concentrations of skim milk powder in water (10%, 5%, and 2½%), in 3 dilutions of butter serum from normal salt free butter, in nutrient broth, and in 0.5% gelatin solution. The resulting emulsions of approximately 32% fat content were pasteurized at 82.2° C. for 10 minutes and, after standing overnight in the ice chest, were inoculated and churned. The butter made from the cream reconstituted in undiluted skim milk (10% skim milk powder) developed surface taint. None of the other butters exhibited the defect.

DISCUSSION

It is recognized, of course, that an odour coming from a skim milk culture of *Ps. putrefaciens* is a blend of different volatile substances. The same holds true for the odour of surface taint from butter. When surface taint is referred to as though it arose from one volatile substance, the principal, essential and controlling component is meant.

There is, to-day, little reason to doubt that *Ps. putrefaciens* is an important cause of surface taint in commercial butter. The evidence is strong, but nevertheless still circumstantial, that the chemical compound imparting the sweaty feet odour to skim milk cultures of the organism is the same compound causing the odour in experimental and commercial surface taint butters. In this study every observation without exception pointed to such identity but final proof is lacking and will probably rest on chemical recognitions.

The observations on the effect of heat treatment on odour production by *Ps. putrefaciens* are, however, important not only in this regard but also in providing a possible explanation for what has been a widely-discussed mystery in Alberta—the sudden and spectacular appearance of the defect in 1919. Just prior to that year there were two significant and province-wide changes in creamery buttermaking practice. Cream neutralization and high-temperature pasteurization for 10 minutes were introduced. There are reasons, not completely authenticated, for believing that the first recognized and recorded case as cited above was not in fact the first outbreak of the defect. In a prior, possible instance coming to our attention the outbreak was immediately preceded by a change to high-temperature pasteurization.

Putrid butter, which may be identical to, or undoubtedly at least includes, the defect known in Canada as surface taint, appears to be increasing in the United States. There seems to be some suggestion that outbreaks follow the institution of high-temperature heat-treatment of the churning cream (11, 12).

It seems highly probable that the following sequence of changes leads to the liberation of the sweaty feet substance from milk and the surface taint substance from butter. A precursor is formed in minute amount in the milk serum under the influence of high temperatures. This precursor appears to follow the casein fraction but the specific relationship is not yet clear. *Ps. putrefaciens* appears to act on this precursor to produce the immediate precursor which may then undergo a chemical change, presumably involving oxidation, to form the sweaty feet and surface taint substance.

SUMMARY

Odour production by *Pseudomonas putrefaciens* in skim milk, whole milk, cream, butter serum, and butter is discussed in relation to surface taint in commercial butter. The relation of odour production to the heat treatment of the milk or cream provides a possible and plausible explanation for the puzzling and sudden appearance of surface taint in Alberta creamery butters in 1919. Attempts to identify the precursor of the surface taint substance were unsuccessful.

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This Series, "Studies on Surface Taint Butter", comprises the following titles which are to be published in *Scientific Agriculture:*

14. I. Odour production by *Pseudomonas putrefaciens.*

15. II. An odourous compound in skim milk cultures of *Pseudomonas putrefaciens.* To be published.

16. III. Some further characteristics of *Pseudomonas putrefaciens.* To be published.

17. IV. Distribution and taxonomy of *Pseudomonas putrefaciens.* To be published.

18. V. The growth of *Pseudomonas putrefaciens* in butter. To be published.

19. VI. Other bacterial species as causal agents. To be published.

STUDIES ON SOME RASPBERRY SOILS OF BRITISH COLUMBIA[1]

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The problem of decline in the growth and production of raspberry plants in certain areas of the Fraser River Valley of British Columbia is one that has been studied from a number of angles during the last few years. Results that have been published to date (2, 3, 4, 5) seem to indicate that there may be a number of factors involved. Harris (4), working at the University of British Columbia, after a number of investigations, has reached the following general conclusion: "The raspberry decline problem in British Columbia is due initially to loss of organic matter (often through faulty management practices) with the resulting loss of ability of the soil to hold nutrients. This results in heavy leaching losses and starvation effects, giving weakened plants. A sulphate deficiency, with its ramifications, is accentuating the trouble . . . . The weakened plants are finally attacked by semi-parasitic root rot fungi of various species (mostly native to the soil) . . . . These fungi will not attack a healthy, vigorous plant, but apparently do attack a plant in a weakened, unhealthy condition, and so contribute finally to its death."

In a later publication by the same author (5) the conclusion is put forward that "the raspberry decline problem in coastal British Columbia in many cases can be attributed to nitrogen, phosphorus, sulphate and potash deficiencies. The first two are the most common. The raspberry responds to luxury feeding of phosphorus in particular." And again, "there seems to be no one cause from which all the declining raspberry plantings are suffering. It is a case of individual diagnosis and remedy for each section, and in many cases for each plantation or part of a plantation. General recommendations are inadvisable."

In 1938, the Division of Horticulture, Central Experimental Farm, brought this problem to the attention of the Division of Chemistry. The latter undertook to carry out some studies in an endeavour to obtain further information about soil conditions in these areas that might throw some additional light on the problem. It was decided to study soil samples that represented a number of areas on which the raspberry growth varied considerably and a suggested sampling program was drawn up. Through the co-operation of the Division of Horticulture and the Dominion Experimental Farm at Agassiz, B.C., the following nine samples were obtained. The terms "poor area" and "good area" refer to areas which gave poor and good raspberry growth respectively.

Sample No. 1. Poor area, from the Henry farm, Hatzic, B.C.; limed at the rate of 5 tons per acre in the fall of 1934; various fertilizer treatments since 1935; 500 pounds per acre $CaCN_2$ in the spring of 1938.

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Sample No. 2. Poor area, from the same farm as sample No. 1; represents an unlimed area adjacent to the limed area; other treatments were identical.

Sample No. 3. Poor area, from the Prosser farm one-quarter mile from the Henry farm; soil cultivated for 40 years and has been in raspberries for 12 years and in orchard previous to that; regularly fertilized with 4–8–10 up to the last 2 or 3 years.

Sample No. 4. Good area, from the Elliot farm adjacent to the Prosser farm where sample No. 3 was taken; this area was in orchard up to about 12 years ago, then in peas and corn; it has been in raspberries for 4 years; no chemical fertilizers have been added, but the area has been regularly treated with barnyard manure.

Sample No. 5. Good area, from the Watson farm about three-quarters of a mile from the Henry farm (samples 1 and 2); this area was planted to raspberries about 5 years ago; a mixture of cow and horse manure has been applied every other year.

Sample No. 6. Good area (in excellent condition) from the McKeown farm about one-half mile from the Henry farm; the planting is about 4 years old and was set out in virgin soil.

Sample No. 7. Very poor area, from the Benbow farm; this area has been in raspberries for 3 years, just previous to which it had been in corn, followed by fall rye; previous to that it had been in blackberries for 20 years.

Sample No. 8. Poor area, from the Cooper farm; this area has been cultivated for about 20 years, growing raspberries chiefly, but it has also been green manured and cropped to potatoes; it has also received applications of lime and fertilizer, but no records of time or rates of application are available.

Sample No. 9. Good area, also from the Cooper farm; raspberry plants were started here 8 years ago on virgin soil; a complete fertilizer (approximately 3–10–8) has been used, but there are no records of amounts applied.

Samples 1 and 2 represented adjacent limed and unlimed areas and both were classed as poor, indicating that liming had no beneficial effect in this case. Samples 3 and 4 represented adjacent poor and good areas, as did samples 8 and 9. The other samples represented areas more widely separated. Of these, sample 6 represented a soil that had been in cultivation for only 4 years, while sample 7 represented an area where almost no growth of raspberries could be obtained.

Some of the individual determinations made on the soil samples from these areas are presented in Table 1. These include pH values, hygroscopic moisture, loss on ignition, carbon, nitrogen, available phosphorus, water-soluble sulphates, water-soluble boron and water-holding capacity.

The pH values were determined by means of the glass electrode, using a soil : water ratio of 1 : 2.5 and taking the reading approximately 15 minutes after mixing the soil and water, with stirring every 5 minutes. There was no apparent relationship between the pH values of the soil samples and good and poor areas. The value for sample 1 (pH 6.65)

reflected the lime treatment that was applied. Sample 8 (pH 6.23) had also received lime at some time. On the other hand, sample 4 (pH 6.29) had a pH value considerably higher than that of the adjacent sample (No. 3, pH 5.29), but no history of liming was given.

Hygroscopic moisture was determined by drying over night in an oven at 100° to 105° C. Loss on ignition was subsequently determined on the same sample, by heating in a muffle furnace at 650° C. for three hours. In the determination of the carbon content of these samples, the dry combustion method was used. The samples were ignited in a silica tube in an electric furnace, using copper oxide as a catalyst; the carbon dioxide was collected in standard $Ba(OH)_2$ and titrated. Nitrogen was determined by the Kjeldahl method.

The variations in hygroscopic moisture and loss on ignition followed the general trend of the organic matter content of these samples as measured by the carbon and nitrogen, showing the lowest values in samples 1, 2 and 8. There was no apparent relationship between the amount of organic matter, as measured by the carbon content, and raspberry growth. The amounts of carbon in the adjacent pair of poor and good samples 3 and 4 were the same (4.15%), but in the other pair of poor and good samples from the Cooper farm (8 and 9), there was considerable difference (2.85% and 4.45%). Furthermore sample 6, the one from soil which had been in cultivation only 4 years and gave excellent growth, had the highest amount of carbon (4.71%) and the second highest amount of nitrogen (0.333%), and sample 7, from a soil that gave very poor growth of raspberry plants, had the highest amount of nitrogen (0.362%) and the third highest amount of carbon (4.37%). The carbon : nitrogen ratio of these soils was higher than the generally accepted value of 10, and ranged from 12.1 to 14.1. The results indicated that these soils were well supplied with nitrogen and organic matter.

Available phosphorus was determined by the method of Truog (14). No apparent connection was found, however, between available phosphorus values and raspberry growth. The lowest and highest values were both from good areas, and the samples from the excellent and very poor areas (6 and 7) had practically the same amount of available phosphorus (42 and 44 p.p.m. P respectively).

Sulphates were measured by extracting the soil samples with water (1 : 5), filtering through a Pasteur-Chamberland filter candle and completing the determination by the benzidine method of Märsden and Pollard (8). No apparent relationship was found between the water soluble sulphates and the level of growth on these soils.

Boron was determined by the method of Naftel (10) in which the soil is extracted with boiling water, and the measurements made colorimetrically using turmeric. In this case there did appear to be some relationship between the results obtained and raspberry growth. All poor areas showed less than 0.20 p.p.m. boron and all good areas showed 0.25 p.p.m. or more, while that marked excellent (No. 6) showed the highest value (0.37 p.p.m. B).

The water-holding capacity of these soil samples was determined by placing 10 grams of the air-dry soil on a moistened filter paper in a funnel, and adding distilled water from a burette drop by drop until the soil was

saturated and a drop came through the neck of the funnel. The amount of water added corresponded to the water held by the soil and since 10 grams of soil were used, the burette reading multiplied by 10 gave the percentage water-holding capacity of the soil. The results obtained indicated that the water-holding capacity of these soils was extremely high (all over 60%). Results obtained for quite a number of soils in this laboratory showed much lower values. Sandy soils showed as low as 30% water-holding capacity and heavy clays about 55%. However, the evidence is not sufficient to establish a relationship between this soil factor and raspberry growth. On the other hand, Harris (5) states that "the results from the field plots definitely indicate that water is probably the chief limiting factor in raspberry plantings in the light, upland soils of British Columbia's coastal regions. Plants which only struggled along without irrigation, flourished luxuriantly under irrigation, with the same fertilizer treatments". The high water-holding capacity which these soils have, may help to account for this fact.

Samples of the soils, approximately 10 pounds each, after being screened through a 2 mm. sieve, were remoistened with distilled water to about 60% of their water-holding capacity and kept in the laboratory at room temperature. After some time (approximately 2 weeks, though this actually varied with different samples from 10 to 16 days), the soils were packed in brass cylinders and the soil solutions displaced according to the method of Burd and Martin (1). Total solids on evaporation were determined by evaporating aliquots in a platinum dish on a steam bath until dry. Total solids on ignition were determined by heating the dried residues in a muffle furnace over night at a temperature not exceeding 425° C. After this ignition, the residues were dissolved in dilute hydrochloric acid, made up to definite volumes in volumetric flasks, and aliquots were analysed for Ca, Mg, K, Na and Fe. Micro-methods were used in analysing for these elements, and the procedures employed by McCance and Shipp (9) were followed. Nitrates were determined on aliquots of the solutions as displaced, using phenol-disulphonic acid. The results obtained are presented in Table 2.

TABLE 1.—RESULTS OBTAINED FROM THE ANALYSIS OF NINE SURFACE SOILS FROM THE HATZIC AREA

Soil number	1	2	3	4	5	6	7	8	9
Description of growth	Poor (limed)	Poor (unlimed)	Poor	Good	Good	Excel-lent	Very poor	Poor	Good
pH values	6.65	5.49	5.29	6.29	5.80	5.82	5.65	6.23	6.00
Percentage hygroscopic moisture	2.97	3.20	3.49	3.60	3.37	3.42	3.99	2.92	3.75
Percentage loss on ignition	7.60	8.11	11.41	11.39	10.80	12.10	11.56	7.75	11.50
Percentage carbon	2.47	2.61	4.15	4.15	4.38	4.71	4.38	2.85	4.45
Percentage nitrogen	0.203	0.206	0.296	0.315	0.310	0.333	0.362	0.214	0.318
C/N ratio	12.2	12.7	14.0	13.2	14.1	14.1	12.1	13.3	14.0
Percentage organic matter (C × 1.724)	4.26	4.50	7.15	7.15	7.55	8.12	7.55	4.91	7.66
P.p.m. available P	64	42	29	58	15	42	44	58	68
P.p.m. sulphates	27	7	16	27	31	21	35	44	44
P.p.m. boron	0.10	0.13	0.04	0.25	0.25	0.37	0.13	0.18	0.25
Percentage water-holding capacity	65.4	65.1	70.3	69.4	72.1	77.8	70.9	60.6	71.1

The concentrations of the solutions obtained by water displacement from the different soils varied considerably, as can be seen from the results for total solids. Both the highest (No. 8) and the lowest (No. 3) concentrations were from soil samples representing areas where raspberry growth was poor, while those from the sample from the area of excellent growth (No. 6) and that from the area of very poor growth (No. 7) were of intermediate concentration. Since the concentration of the soil solution is largely biologically controlled, these varying results may be due in part to the different microbiological activities of the samples concerned. The concentration of nitrates followed the same general trend as that of the total solids in the solutions.

In order to obtain a basis for comparison, the values for Ca, Mg, Na and Fe were recalculated on the basis of 1000 p.p.m. total solids on ignition, the figures for the results being given in the bottom part of Table 2. No results for potassium are presented. Unfortunately, while carrying out this determination, the samples became contaminated, and it was later found that insufficient material was available to repeat the determination.

TABLE 2.—RESULTS OF THE ANALYSIS OF THE DISPLACED SOIL SOLUTIONS

(Expressed in p.p.m. displaced solution)

Soil number	1	2	3	4	5	6	7	8	9
Description of growth	Poor (limed)	Poor (unlimed)	Poor	Good	Good	Excellent	Very poor	Poor	Good
Total solids on evaporation	1506	1392	900	1490	1110	1531	1298	2003	1272
Total solids on ignition	1018	938	596	1178	580	769	666	1412	865
Loss on ignition	488	454	304	312	530	762	632	591	407
NO_3	824	832	498	813	718	984	765	1012	748
Ca	292	243	161	279	151	208	200	361	227
Mg	6	18	13	19	30	41	30	14	12
Na	3	6	6	6	19	14	9	21	7
Fe	0.25	0.13	–	0.18	0.17	0.16	0.27	0.51	0.14
Results calculated on the basis of 1000 p.p.m. total solids on ignition									
Ca	287	262	270	237	261	270	300	256	262
Mg	6	19	22	16	52	52	45	10	14
Na	3	6	10	5	33	18	15	15	8
Fe	0.25	0.14	–	0.15	0.29	0.21	0.41	0.36	0.16

Results for calcium showed less variation than did those for the other elements. The highest value appeared in sample 7 (from the very poor area). Magnesium results were highest in samples 5, 6 and 7 (from good, excellent and very poor areas) and were lowest in those samples (Nos. 1 and 8) where lime had been applied to the soil. Values for sodium and iron showed considerable variation but no relationship with the growth of raspberries.

Exchangeable bases were determined in these soil samples by leaching with neutral normal ammonium acetate. An aliquot of the leachate was titrated potentiometrically with standard NH_4OH to pH 7 and the

results calculated to exchangeable hydrogen. The rest of the leachate was taken to dryness and ignited to remove organic matter, and the residue taken up in dilute HCl. The bases in this solution were then determined by standard methods. In the case of iron and aluminum, these were determined together by precipitating as phosphates, redissolving and determining the phosphorus colorimetrically; the iron was determined colorimetrically as the thiocyanate and aluminum was obtained by difference. The results obtained are presented in Table 3.

TABLE 3.—RESULTS OBTAINED FOR THE EXCHANGEABLE BASES

(Expressed in milliequivalents per 100 g. dry soil)

Soil number	1	2	3	4	5	6	7	8	9
Description of growth	Poor (limed)	Poor (unlimed)	Poor	Good	Good	Excel-lent	Very poor	Poor	Good
H	2.29	6.92	10.11	5.00	7.09	9.19	9.04	4.52	8.00
Ca	11.52	4.19	3.51	12.39	7.73	7.55	7.69	8.24	10.13
Mg	.07	.13	.21	.49	.90	1.33	.63	.11	.23
K	.24	.33	.30	.50	.53	.52	.49	.41	.36
Na	.11	.19	.12	.15	.13	.17	.14	.17	.19
Fe	.006	.009	.011	.012	.009	.016	.011	.007	.016
Al	.015	.073	.386	.048	.159	.134	.097	.051	.109
Mn	.011	.045	.065	.033	.046	.105	.090	.020	.070
Total exchangeable ions	14.262	11.887	14.712	18.623	16.594	19.015	18.188	13.528	19.105

As was to be expected, there was a general agreement between exchangeable hydrogen, exchangeable calcium, and pH values; as the pH values decreased, exchangeable hydrogen increased and exchangeable calcium decreased. This was more clearly shown when these exchangeable ions were calculated as percentage of the total exchangeables. In the case of hydrogen, this figure represents the percentage unsaturation. These figures are presented in Table 4, the soil samples being arranged in order of decreasing pH values. It is thus clearly seen that (with the one exception of sample 5), the percentage of unsaturation increased regularly, and (with the one exception of sample 6) the percentage of calcium in the total of exchangeable ions decreased regularly, with decreasing pH values.

TABLE 4.—COMPARISON OF pH VALUES, PERCENTAGE OF UNSATURATION AND PERCENTAGE OF Ca IN TOTAL EXCHANGEABLE IONS

(Arranged in order of decreasing pH values)

Soil Number	1	4	8	9	6	5	7	2	3
Description of growth	Poor (limed)	Good	Poor	Good	Excel-lent	Good	Very poor	Poor (unlimed)	Poor
pH value	6.65	6.29	6.23	6.00	5.82	5.80	5.65	5.49	5.29
Percentage of unsaturation	16.1	26.8	33.4	41.9	48.3	42.8	49.6	58.1	68.7
Percentage of calcium	80.7	66.3	60.9	53.0	39.7	46.6	42.3	35.3	23.8

In general, also, exchangeable aluminum was high at low pH values, and low at high pH values. Available manganese was also decreased by liming, as shown by the low values for exchangeable Mn in samples 1 and 8, both of which had received lime, and in sample 4 which had a high pH value though no history of liming was given. There was, however, no indication that the amount of any of the exchangeable bases was related to the level of raspberry growth on the areas represented by the samples.

An attempt was made to obtain some data on the constitution of the organic matter of these soils. A system for the proximate analysis of the organic matter of mineral soils has been proposed by Waksman and Stevens (15) and modifications of this procedure have been used by Shewan (13) and by Salisbury and De Long (11). The procedure adopted in the work reported herein was briefly as follows:

(a) A 50-gram sample of soil was extracted in a Soxhlet apparatus for about 30 hours with a mixture of benzene and alcohol. The extract was evaporated to dryness in a weighed dish.

(b) The soil sample from the above was allowed to dry in the Soxhlet thimble over night at room temperature, then for 2 to 3 hours at 105° C. It was then transferred to a 2-litre flask, 200 cc. distilled water added, and the whole refluxed for 2 hours. It was then filtered through a Buchner funnel and washed with hot water to a volume of 1 litre. Total nitrogen was determined on a 200 cc. aliquot, and total organic matter on a 500 cc. aliquot by taking to dryness, weighing, igniting and weighing.

(c) After being dried, the residue was returned to a 2-litre flask, 700 cc. of 2% HCl added, and the whole refluxed for 5 hours. It was then filtered through a Buchner funnel and washed with cold water to a volume of 2 litres. Total nitrogen was determined on a 200 cc. aliquot and amide nitrogen on a 200 cc. aliquot. Reducing sugars were measured by the Lane and Eynon method (7) on aliquots of 40 cc. which were neutralized with 2.5% NaOH solution, filtered and diluted to 100 cc. As standard, a solution of glucose containing 1.6736 grams per litre was used.

(d) The residue from *(c)* was dried and transferred to a 500 cc. Erlenmeyer flask. Approximately 75 cc. of cool 80% H_2SO_4 were added and the mixture kept at about 5° C. for 2½ hours. It was then transferred to a 2-litre flask with 1125 cc. distilled water, refluxed for 5 hours and let stand over night. It was filtered in the morning and washed with cold water to a volume of 2 litres. Total nitrogen was determined on a 200 cc. aliquot, as after HCl hydrolysis, and reducing sugars were determined on a 40 cc. aliquot which in this case was neutralized with 20% NaOH, filtered and made to 100 cc.

(e) Total carbon and nitrogen were determined on the dried residue by the dry combustion and Kjeldahl methods, respectively.

The results obtained, calculated as percentage of the total organic matter of the soil (% C × 1.724), are presented in Table 5. It is seen that these fractions accounted for, on the average, about 85% of the total organic matter. This figure was slightly greater than the average of about 80% obtained by Salisbury and De Long (11), but less than the average of about 92% obtained by Waksman and Stevens (15) and that of about 88% (for the A_1, A_2, and B_1 horizons) taken from the results by Shewan (13). In general the ligno-humus complex represented the greatest fraction of the organic matter, followed in order of magnitude by the protein fraction, the hemicelluloses and the celluloses. These results also correspond with those found by other workers.

TABLE 5.—ORGANIC MATTER FRACTIONS AS PERCENTAGE OF SOIL ORGANIC MATTER

Soil number	1	2	3	4	5	6	7	8	9
Description of growth	Poor (limed)	Poor (unlimed)	Poor	Good	Good	Excel-lent	Very poor	Poor	Good
Alcohol-benzene extract	1.37	1.48	2.69	1.54	1.57	2.76	1.82	1.38	2.07
Water extract	7.10	5.36	4.07	4.01	3.59	5.85	4.47	4.28	4.77
Hemicelluloses	5.71	10.64	10.30	10.38	9.52	10.88	11.40	12.38	9.55
Celluloses	1.92	5.52	4.37	3.67	3.58	5.01	4.57	5.12	5.44
Protein	27.88	27.22	24.50	26.43	23.82	24.91	29.82	25.28	25.40
Ligno-humus	30.15	34.65	40.45	35.40	39.55	40.95	36.90	37.85	43.80
Percentage accounted for	74.13	84.87	86.38	81.43	81.63	90.36	88.98	86.29	91.03

There was nothing in the figures to represent any consistent differences between samples from good and poor areas. Nor did the results for sample 6, which had been in cultivation for only 4 years and produced excellent crops of raspberries, indicate that its organic matter fraction differed very much in composition from that of other samples; its results appeared to correspond more nearly with those for sample 3 from a poor area which had been in cultivation for about 40 years, and sample 9 from a good area which had been cultivated about 8 years.

The distribution of nitrogen in the various fractions is shown by the figures presented in Table 6. The results are expressed as percentage of the nitrogen in the original soil. The amount accounted for in the different fractions was always within 5% of the total amount present in the original sample, and in many cases it was even closer to 100%. There was no apparent distribution of nitrogen which could be related to the growth of raspberries on the areas represented by the soil samples.

TABLE 6.—DISTRIBUTION OF NITROGEN IN THE ORGANIC MATTER FRACTIONS

(Expressed as percentage of nitrogen in original sample)

Soil number	1	2	3	4	5	6	7	8	9
Description of growth	Poor (limed)	Poor (unlimed)	Poor	Good	Good	Excel-lent	Very poor	Poor	Good
Water-soluble	6.9	4.9	3.7	3.8	3.2	5.1	3.3	2.3	4.4
HCl-soluble-amide	13.8	12.1	12.2	13.0	12.6	11.4	10.8	12.1	12.3
HCl-soluble-non-amide	38.4	39.8	38.5	39.7	36.8	35.7	40.9	36.9	39.0
H_2SO_4-soluble	13.8	13.1	14.2	14.9	12.9	16.8	16.9	13.5	13.5
In residue	27.8	30.1	30.0	28.6	30.6	33.3	31.2	30.4	33.3
Percentage recovery	100.7	100.0	98.6	100.0	96.1	102.3	103.1	95.2	102.5

It had been observed (4) that greatly improved growth of raspberry canes could be obtained if the soil in which the growth was poor was steam sterilized. To study this point, a part of each of these 9 soil samples was heated in an autoclave at 15 pounds pressure for 3 hours. They were then examined and results compared with those obtained for the unsterilized soil samples. Some outstanding differences were observed. In the first

place, the solubility of the organic matter was noticeably increased. This was shown by the results for the total solids on evaporation and ignition of the displaced soil solutions, as presented in Table 7.

Whereas the total solids on ignition were greater than the loss on ignition in the solutions from the unsterilized soils, in most cases twice as great, this relationship was reversed in the case of the solutions from the sterilized soils and the loss on ignition in a number of cases was several times the total solids on ignition. This evidence of increase in soluble organic matter was supported by the results obtained in the fractionation of organic matter. The water-soluble organic matter was greater in the sterilized than in the unsterilized soils (Table 7). The nitrogen in the water soluble extracts, expressed as a percentage of the total nitrogen of the soils, was also greater in the sterilized soils. It is interesting to compare the percentage increase of the water soluble nitrogen in soils from

TABLE 7.—SOME COMPARISONS OF STERILIZED AND UNSTERILIZED SOILS

Soil number	1	2	3	4	5	6	7	8	9
Description of growth	Poor (limed)	Poor (unlimed)	Poor	Good	Good	Excel-lent	Very poor	Poor	Good
Total solids on evaporation*									
Unsterilized	1506	1392	900	1490	1110	1531	1298	2003	1272
Sterilized	2227	2259	2198	2860	2275	2672	3336	2331	2418
Total solids on ignition*									
Unsterilized	1018	938	596	1178	580	769	666	1412	865
Sterilized	867	485	265	661	415	322	510	813	475
Loss on ignition*									
Unsterilized	488	454	304	312	530	762	632	591	407
Sterilized	1360	1774	1933	2199	1860	2350	2826	1518	1943
Water-soluble organic matter†									
Unsterilized	7.10	5.36	4.07	4.01	3.59	5.85	4.47	4.28	4.77
Sterilized	8.41	7.42	5.74	5.64	5.20	7.41	6.90	4.92	6.19
Nitrogen in water-soluble organic matter‡									
Unsterilized	6 9	4.9	3.7	3.8	3.2	5.1	3.3	2.3	4.4
Sterilized	9.0	7.8	7.2	6.0	6 0	7.5	7.0	4.8	7.0
Percentage increase in water-soluble N in sterilized soils	30.5	59.2	94.7	58.0	87.5	47 0	112.0	109.0	59 1
Water-soluble sulphates**									
Unsterilized	27	7	16	27	31	21	35	44	44
Sterilized	51	41	62	63	62	61	79	70	74
Exchangeable manganese††									
Unsterilized	.011	.045	.065	.033	.046	.105	.090	.020	.070
Sterilized	.460	1.149	1.369	.726	.906	.977	1.408	.570	.952

* P.p.m. displaced solution.
† Percentage total organic matter.
‡ Percentage total N.
** P.p.m. air-dry soil.
†† M.e. per 100 g. dry soil.

poor and good areas. In three comparisons, there was a greater proportionate increase in the water-soluble nitrogen of the soils from poor areas. For instance, sample 3 (poor) showed an increase of 94.7% while sample 4 (good) from an adjacent farm showed an increase of only 58.0%. Again, sample 8 (poor) showed an increase of 109% while sample 9 (good) from the same farm showed an increase of 59.1%. Finally, sample 7, the soil from the very poor area, showed an increase in water-soluble nitrogen of 112% while that from the excellent area, sample 6, showed an increase of only 47%. However, on the other hand, samples 1 and 2, limed and unlimed, but both from areas classified as poor, showed smaller increases than the other samples from poor areas (30.5% and 59.2%, respectively), but it will be observed that both of these were comparatively well supplied with water-soluble organic matter and nitrogen.

Two other outstanding differences were observed between sterilized and unsterilized soils. The water-soluble sulphates and exchangeable manganese were both markedly increased by sterilization (Table 7).

The fact that sterilization of a soil brings about chemical and physical, as well as biological changes, has been recognized for a considerable time. Kopeloff and Coleman (6) have presented a comprehensive review of the literature. It is generally recognized that the solubility of various constituents, chiefly the organic matter, is increased by sterilization. In the case of the soils under discussion in this paper, the increase in the solubility of the organic matter has been noted, as well as the availability of sulphates and manganese. From other results obtained but not presented in the foregoing, there was no evidence that the availability of calcium or potassium was increased, though in most cases a slight increase in the availability of magnesium and iron was indicated. The availability of phosphorus as measured by Truog's method was decreased in all cases. Though the acidity of the soils as measured by the pH values did not appear to be greatly changed by sterilization, there was in every case a decrease in the exchangeable hydrogen with, at the same time, a decrease in the exchangeable aluminum. All sterilized soils were much more difficult to "wet" than the unsterilized soils.

In connection with the increased solubility of organic matter and nitrogen by sterilization, Schreiner and Lathrop (12) have presented evidence to show that such nitrogen-containing compounds as xanthine, hypoxanthine, guanine, cytosine and arginine were formed, or their quantity in the soil increased, by heating. These compounds were found to be beneficial to plant growth. Sterilization of the soil samples has brought about an increase in the soluble organic matter and nitrogen with an indication in some cases that there was a greater proportional increase in the availability of the nitrogen in the soil samples from areas of poor raspberry growth. At the beginning of this investigation, it was suggested by one of the field workers that the nitrogen status of these soils was probably important. Such considerations indicate that a more intensive study of the nitrogen fractions might well be undertaken in order to throw some further light on the composition of these soils and their ability to support the growth and production of raspberries.

SUMMARY

This investigation dealt with a study of a number of soil samples from areas which showed varying levels of growth of raspberries. Some of the factors examined were pH values, carbon and nitrogen contents, available phosphorus, water-soluble sulphates, water-soluble boron, water-holding capacity, exchangeable bases, displaced soil solution, proximate analysis of the organic matter fraction, and the effect of sterilization on most of these values.

The soils appeared to be well supplied with organic matter. Their water-holding capacity was quite high for mineral soils. Of the factors studied, the only one that seemed to show a relationship with the level of raspberry growth was the boron content of the soil samples. The samples from all the areas showing good growth had higher boron contents than those from the areas of poor growth, and the sample from the area where excellent raspberry growth had been obtained had the highest boron content of all. It has been shown that the beneficial effects of sterilization of the soils may be due to a number of factors.

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A COMPARATIVE STUDY OF THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF CERTAIN SAWFLIES AFTER HIBERNATION IN THE COCOON[1]

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For some time Canadian entomologists have become increasingly aware of the importance of sawflies attacking forest and shade trees. A considerable amount of time and energy has been devoted to the study of these insects, especially in connection with the Dominion Forest Insect Survey, a co-operative project in which some 2000 observers throughout Canada participate by collecting samples of forest insects in their particular district and forwarding them to the nearest forest insect laboratory for identification and study. The large-scale rearing experiments with overwintering cocoons conducted annually in several Canadian laboratories must be completed on schedule to forestall interference with the next year's program of activities, and the success of these experiments naturally depends in a large measure on a knowledge of optimum conditions for development of the insects in the incubator. It became imperative, therefore, to determine the characteristic reaction of the various species concerned to the conditions of temperature and humidity to which they are subjected in the laboratory.

With this object in mind, the present study of several species of sawflies was undertaken. It should be understood that the data presented here do not constitute a thorough, intensive study of temperature relationships, but rather the results of a comparatively easy method of determining, for practical purposes only, the characteristic rates of development of the various species when subjected to certain controlled conditions of temperature and humidity. On the other hand, it will be shown that, in spite of the admitted imperfections of the experimental methods, these relationships determined under laboratory conditions resemble those obtaining in nature closely enough to afford at least a partial explanation of related phenomena, such as distribution and seasonal development, observed in the field. As such, they may lay claim to a certain comparative value.

It is fully realized, with Shelford (8) and later workers (4, 6, 7), that the true rate of development of a living organism cannot be expressed adequately by means of a straight line (being really a sigmoid curve), and that its thermal requirements cannot be accurately described in day-degrees. At the same time, it is felt that for practical purposes and for medial temperatures the acceptance of the linear relationship and the day-degree concept is justified, and that the essential temperature characteristics of these sawfly cocoons may be expressed, according to the method of Glenn (3), by means of a Thermal Constant (or sum of effective temperatures) and a Theoretical Threshold of Development. It is believed

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that these values can be relied upon by subsequent workers to obtain an idea of the rate of development at any medial temperature, whether in a thermostabilized incubator or in the field.

The results obtained in the course of this study represent 2 years of experimental work with some 10,000 cocoons of 8 species. They are by no means final, but it is hoped that they may be useful as a basis for further comparative studies of thermal requirements. In addition to those discussed below, a few other species were studied, but the results obtained were discarded, because they were either quantitatively insufficient or based on faulty initial technique. In the case of the Diprionine sawflies, the stage within the cocoon at the time of transfer to effective temperature —whether eonymph or pronymph—was not considered, the aim being to portray average conditions by using large quantities of material.

METHODS

The sources of the material used were as follows:

Neodiprion pinetum Norton: Thessalon, Ontario.
Neodiprion swainei Midd.: Laniel, Quebec.
Neodiprion lecontei Fitch: Lachute, Quebec, and Thessalon, Ontario.
Neodiprion dubiosus Schedl: Various localities in Eastern Canada, from Forest Insect Survey collections.
Pikonema dimmockii Cresson: Various localities in Eastern Canada, from Forest Insect Survey collections.
Pikonema alaskensis Rohwer: Lanoraie, Quebec.
Pristiphora geniculata Htg.: Berthierville, Quebec.
Pristiphora erichsonii Htg.: Labelle County, Quebec.

The cocoons passed the winter in a cold cellar, being subjected to a temperature of approximately 0° C. for 3 months. At the beginning of the experiments, they were warmed at the rate of 1 degree per hour, and on reaching successive points on the temperature gradient, were put into the appropriate chambers in a multiple temperature incubator.

This apparatus was similar to that described by Zwölfer (9), being essentially a large copper U-tube between a source of heat and a vessel of melting ice, forming the top, bottom and back of the chambers successively spaced along its length. The source of heat was a tank ($1\frac{1}{2}$ ft. square) of hot water kept at 45° C. by 2 electric heaters (G.E. Calrod), controlled within 0.2° C. by a bi-metallic double-pole DeKhotinsky thermoregulator, and recorded by a Tycos recording thermometer. The copper U-tube was 140 inches long, 9 inches at either end being inserted into the hot tank and ice box, thus enclosing 10 chambers each 14 in. long, 10 in. high and 14 in. deep. The ice box was replenished twice a day. The metal part of the apparatus was insulated by a thickness of four inches of regranulated cork. The temperature in each chamber was checked 3 times daily for a period of 1 month immediately previous to the experiment, and a thermometer was kept in each chamber throughout the experiment. Fluctuations were not great, seldom ranging more than ± 0.5° C.

The cocoons were handled in $\frac{1}{3}$-pint glass jelly jars $2\frac{1}{2}$ in. in diameter and 3 in. high. By means of a saturated solution of potassium nitrate on the bottom of the jar, a controlled humidity of 90 to 95% could be

obtained. The cocoons were suspended above the salt on a disc of copper screening fixed by a rim of celluloid. Vaseline prevented creeping of the solution. An air-tight metal top was employed, a small hole (with a cork) being used for withdrawal of emerged adults. Up to 50 cocoons were placed in each jar. The material was examined for emergence 3 times a day: 8 a.m., 2 p.m., and 6 p.m. Emergents were removed by suction through the hole in the lid of the jar.

In 1939, when this study was first incepted, no attempt was made to control humidity in a systematic way, and relative humidities averaged about 40 to 45%. Therefore, those results were treated as purely exploratory and were discarded. However, for sake of comparison, the results for *Neodiprion lecontei* Fitch and *Pristiphora geniculata* Htg. under these dry conditions are included in the tables and graphs.

TREATMENT OF RESULTS

From the emergence data, the length of time in the incubator was calculated for each emergent in hours[4]. (There would be a slight error on the plus side in some cases due to the interval between observations.) From this, the average number of days required for emergence was calculated for each temperature group, males and females separately.

That the number of cocoons used was large enough to prevent minor variations or occasional errors of observation from affecting general results can be seen from Table 1, where standard deviations, as compared with the mean values, are summarized.

TABLE 1.—STANDARD DEVIATIONS IN RELATION TO POPULATION MEANS, IN DAYS

Species		25°		22°		19°		16°		13°	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Neodiprion lecontei Fitch.	♀	15.1	1.4	17.9	1.5	24.9	1.7	33.2	2.9	72.7	4.7
	♂	15.1	1.8	17.7	—	23.4	3.8	29.7	3.6	59.2	11.0
Neodiprion swainei Midd.	♀	23.6	1.8	28.1	4.4	33.6	6.3	48.4	9.4	113.6	24.7
	♂	23.1	3.6	27.3	4.2	31.2	8.2	51.6	9.0	102.5	30.0
Pristiphora erichsonii Htg.	♀	—	—	24.8	—	39.0	6.0	64.0	24.5	105.9	26.7
Pristiphora geniculata Htg.	♀	33.2	—	36.7	2.2	41.1	2.8	43.4	6.3	69.7	7.1
	♂	—	—	36.8	0.8	39.5	2.8	43.2	3.7	68.9	5.8
Pikonema alaskensis Roh.	♀	29.9	2.8	32.7	2.7	40.6	4.0	55.1	8.9	82.6	13.8
	♂	26.8	—	31.9	5.4	38.5	10.9	50.0	9.1	70.3	15.8

From the average time taken for emergence in each temperature group, the developmental index was derived as the reciprocal. Then, for each species, the developmental indices at each temperature were plotted on a graph against temperature as the ordinate.

[4] Slight additional time was arbitrarily added in each case to take care of effective temperature afforded during the conditioning period.

The linear relationship characterizing these points on the graph was calculated by the method of least squares. A special modification was made in that each point was weighted by the number of observations it represented. The method was taken from an analogous procedure in forest mensuration (1) and is in essence as follows:

The linear relationship is expressed by the equation

$$X = a(A) + k,$$

where X = Developmental Index,

A = Temperature,

a and k are constants to be determined.

The required values for a and k are obtained by substituting and solving in the following Normal Equations:—

$$\Sigma(fA^2)a + \Sigma(fAK)k - \Sigma(fAX) = 0$$
$$\Sigma(fAK)a + \Sigma(fK^2)k - \Sigma(fKX) = 0$$

where K is unity

f = frequency of observations.

It should be noted at this point that there is a decrease in critical increments at temperatures above 25° C. This falling off from the linear relationship at higher temperatures is especially noticeable in the graphs for *Neodiprion swainei* Midd. and *N. lecontei* Fitch. Therefore, it was considered advisable to limit the calculation of the thermal constant relationship to temperatures between 25° C. and the threshold; although the points at higher temperatures are plotted on the graph. Similarly, in the lower ranges of temperature, approaching the actual threshold, critical increments are again less than at medial temperatures—a symptom of the really sigmoid character of the relationship. However, since for practical purposes the threshold is useful only to determine the effective temperature in the truly developmental ranges, this minor deviation is ignored. Therefore, the point at which the calculated index line crosses the X axis is taken as the theoretical threshold: this figure, subtracted from the actual temperature, gives the effective temperature.

By reference to the index line, the developmental index at any effective temperature may be read, and from it the time required to produce emergence may be calculated as its reciprocal. The product of this time in days and the effective temperature in degrees gives the thermal constant for the species in day-degrees.

RESULTS

The results of this investigation are summarized in the series of graphs for each of the 8 species studied (Figures 1 and 2). The average values at each temperature from which the index lines are calculated have been inserted, those for males marked by oblique crosses, for females by vertical crosses, and for each value the number of observations is indicated. To broaden the general picture, percentage of emergence at the different temperatures is also indicated. To render comparison easier, the values for the thermal constant and the theoretical threshold are summarized in Table 2. In addition, the expected periods of time required to produce emergence at 2 incubator temperatures, 20° C. and 25° C., have been calculated from the graphs. Values for these periods may be found in the last 2 columns.

TABLE 2.—THEORETICAL THRESHOLDS AND THERMAL CONSTANTS FOR THE DIFFERENT SPECIES

Species		Number of observations	Theoretical threshold	Thermal constant	Time for development	
					20° C. = 68° F.	25° C. = 77° F.
			°C.	day-degrees	days	days
A—under controlled conditions of humidity (90–95% R.H.)						
Neodiprion dubiosus Schedl	♂	19	10.9	157	17.2	11.2
	♀	38	10.9	157	17.2	11.2
Neodiprion lecontei Fitch	♂	38	8.4	245	21.2	14.8
	♀	223	9.5	230	22.0	14.9
Neodiprion pinetum Nort.	♂	16	8.5	225	19.6	13.7
	♀	32	6.6	303	22.6	16.5
Neodiprion swainei Midd.	♂	99	9.1	352	32.3	22.0
	♀	176	9.0	365	33.2	22.8
Pristiphora erichsonii Htg.	♀	81	9.8	358	35.4	23.6
Pristiphora geniculata Htg.	♂	260	1.4	709	38.2	30.0
	♀	178	1.7	714	39.1	30.7
Pikonema dimmockii Cress.	♀	18	0.7	532	27.6	21.8
Pikonema alaskensis Roh.	♂	73	1.3	680	36.4	28.7
	♀	341	1.3	730	38.9	30.8
B—under "dry conditions" (40–45% R.H.)						
Neodiprion lecontei Fitch	♂	115	9.4	337	31.8	21.6
	♀	118	10.8	289	31.8	20.4
Pristiphora geniculata Htg.	♂	58	−39.0	2680	45.3	41.8
	♀	142	−10.0	1290	43.0	36.8

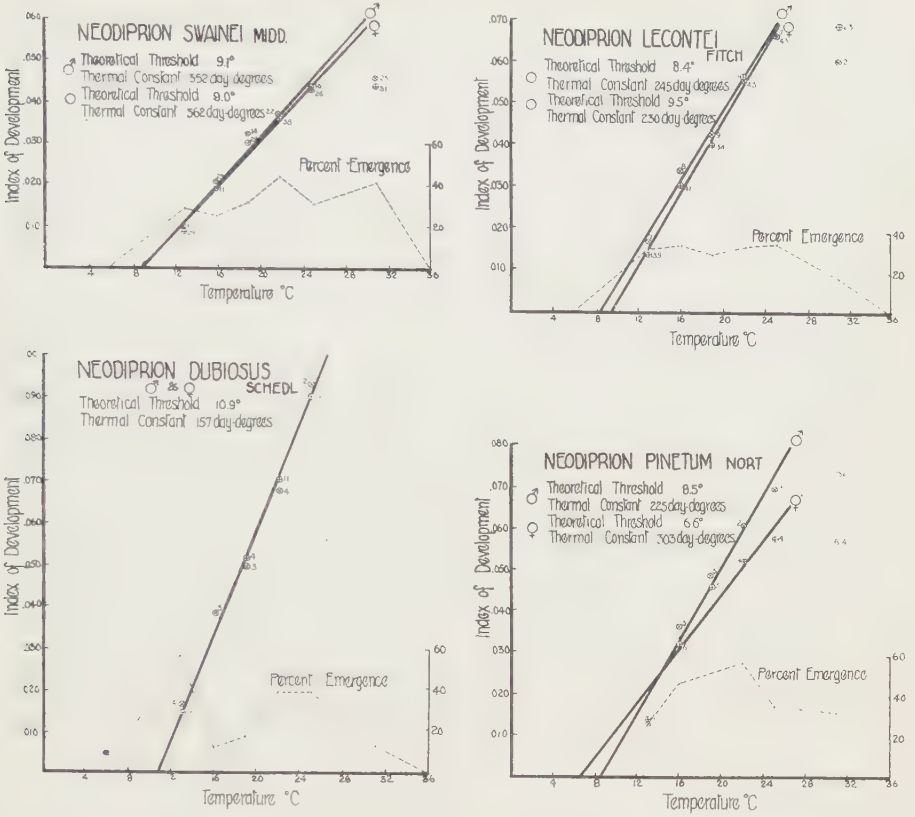
Analysis of the figures above and of the graphs seems to warrant the following conclusions:

1. In most cases, the value of the thermal constant is slightly higher for females than for males. Values for the theoretical threshold are nearly the same for both sexes, varying either way.

2. As a class, the genus *Neodiprion* is characterized by a high theoretical threshold and a comparatively low thermal constant. In other words, this group requires higher temperature to start development, but emergence follows in a relatively shorter time.

3. The highest theoretical thresholds—and lowest thermal constants—are exhibited by *Neodiprion lecontei* Fitch and *N. dubiosus* Schedl. Both of these species show a preference in the field for open-grown trees, where the ground is rapidly heated in the spring by insolation. This consideration is further emphasized by a comparison of *N. dubiosus* Schedl and *N. swainei* Midd. While the former typically attacks isolated trees in the open, the latter, with a lower theoretical threshold, is more generally found in jack pine stands in the forest.

4. The optimum temperature, as far as survival is concerned, lies in the vicinity of 22° C. (72° F.) for the species of *Neodiprion* studied; for those of the genera *Pristiphora* and *Pikonema*, it is in general lower.



5. By contrast, the genus *Pikonema* is characterized by a low theoretical threshold and a high thermal constant. Indeed, the theoretical threshold of *P. dimmockii* is less than 1 degree above the freezing point, and in this connection its northern distribution and consistent occurrence in cool spruce woods, as revealed by the records of the Forest Insect Survey, is noteworthy.

6. Cocoons of *Pristiphora geniculata* have temperature characteristics resembling those of *Pikonema*. But the material of *P. erichsonii*, perhaps in contrast to some established opinions, exhibited the high theoretical threshold and lower thermal constant characteristic of *Neodiprion*.

7. The effect of a controlled high humidity is to be found in:

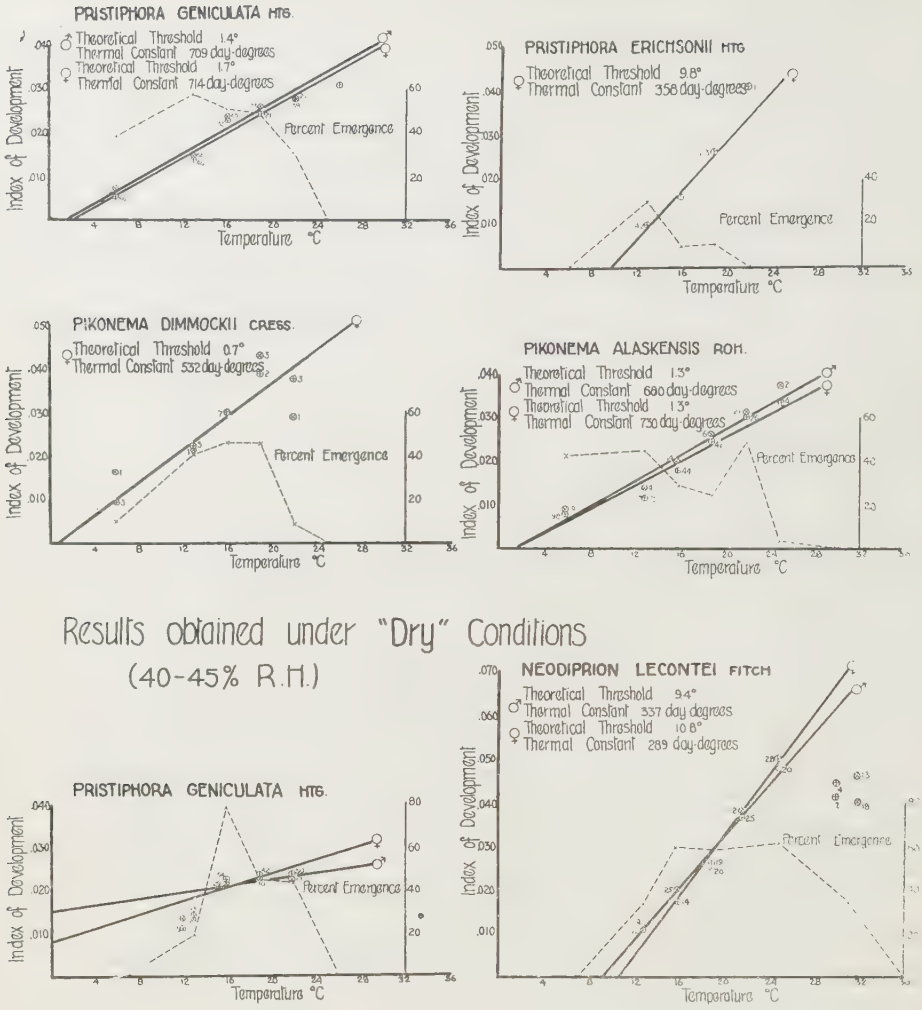
(a) better results; *vide* the bewildering index lines for *P. geniculata* under dry conditions.

(b) a lower thermal constant; dry conditions tend to retard development.

(c) increased mortality; in 6 of the 8 species, survival was higher at 40–50% R.H. than at 90–95% R.H.

INTERPRETATION OF FIELD OBSERVATIONS

In the course of some experiments carried on for several years, a large number of observations were made on the dates of emergence of sawfly



cocoons in the field. The material was kept outdoors over the winter and spring in screened cages simulating natural conditions at ground level. Table 3 gives the dates when 50% of the adult emergence was obtained.

TABLE 3.—DATES WHEN 50% EMERGENCE OCCURRED IN THE FIELD

	1936	1937	1938	1939	1940
Neodiprion lecontei Fitch	—	June 11	June 18–19	June 20	—
Neodiprion swainei Midd.	—	June 7	—	—	—
Pristiphora erichsonii Htg.	—	—	—	—	June 2– 3
Pristiphora geniculata Htg.	June 5–6	June 6–7	June 9	June 17–18	June 11–12
Pikonema alaskensis Roh.	—	—	—	—	May 31 – June 1

From Table 3, it can be seen that a species like *Pristiphora geniculata*, with a handicap of a high thermal constant, can emerge at an earlier date than *Neodiprion lecontei*, which has a very much lower thermal

constant. This is attributable to the fact that *P. geniculata* has a much lower threshold, and can thus develop at temperatures where *N. lecontei* is still dormant. It is evident that in a climate such as that of Berthierville, Quebec, where spring ground temperatures are between 0° and 10° C. for a long period, a species with a threshold of about 1° C. can utilize sufficient effective heat to enable it to overcome the handicap of requiring a high sum of effective temperature.

These results also offer an opportunity of making a comparison between the thermal constant of a species in the field and that obtained under constant temperature and humidity in the laboratory.

Effective air temperatures operating in the field, up to the time of emergence, were calculated from thermograph records, taken at the 4-foot level[5]. Readings of temperature were recorded every 2 hours and the theoretical threshold value subtracted therefrom. The effective temperature for each day was calculated by taking the sum of the 2-hour readings above the threshold and dividing by 12. These effective temperatures, operating from the opening of the spring season up to the date of 50% emergence, were then totalled to give the thermal constant for the species in day-degrees. These values could then be compared with those obtained from the laboratory experiments, as in Table 4.

TABLE 4.—COMPARISON OF VELOCITY OF DEVELOPMENT IN THE FIELD AND IN THE LABORATORY

Species		Number of observations	Thermal constant in field	Thermal constant in lab'ry	Percentage decrease in thermal constant in field
			day-degrees	day-degrees	
Neodiprion lecontei Fitch	♀	1979	224	230	2.6
	♂	3292	240	245	2.0
Neodiprion swainei Midd.	♀	38	305	365	16.4
	♂	18	301	352	14.5
Pristiphora geniculata Htg.	♀	4648	575	714	19.5
	♂	2100	561	709	20.0
Pristiphora erichsonii Htg.	♀	173	318	358	11.2

[5] Temperatures in the cages were lower, but approximated the air temperature rather than the soil temperature once the snow had disappeared.

Although there is some measure of agreement between the field and the laboratory values, those in the field are generally considerably lower. This may be explained, in part, by the stimulating effect of variable temperature (2, 4, 5, 6, 8). Probably a more potent cause of this difference lies in the discrepancy between theoretical and actual threshold, since some slow development may take place at temperatures below the point calculated as the theoretical threshold.

ACKNOWLEDGMENTS

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HYBRID CORN STUDIES I

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Five years ago very few corn growers in the Dominion of Canada were even mildly interested in hybrid corn. Today, most producers of seed corn and many users of this product are vitally concerned with hybrid corn in all its phases. Such evidence of increased interest in any crop must be based on some peculiarities of growth that increase its value to the farmer. The object of the present paper is to present a few results of studies contrasting some of the better hybrid corns with a few commonly grown varieties. The characters reported on are yield, root development, strength of stalk, breaking strength of internodes, stalk structure, and tolerance to corn borer.

MATERIALS AND METHODS

The hybrids chosen were selected from the two earlier maturity groups recommended by the Ontario Committee on Hybrid Corn. These hybrids were compared with three of the most commonly grown standard varieties. Samples were taken in all cases when the corn was approaching the glazed stage of maturity.

The corn was grown in all cases on the experimental grounds of the Ontario Agricultural College. The soil on the experimental area is classified as Guelph loam, and is in a fair state of fertility. Hill planting was used throughout, the hills being 3 feet apart each way with 3 stalks per hill; 4 plantings were made of each lot.

The assistance of the Department of Agricultural Engineering was secured in developing apparatus suitable for the measurement of the relative resistance of the internodes of the different lots of corn to breaking, crushing, and penetration. A machine lathe was adapted to provide a uniform pull. Between the point of attachment on the movable carriage of the lathe and a stationary point which served as an anchorage, a dynamometer was installed to register, in pounds, the pull exerted in the various types of tests. The breaking point was determined by placing the internodal lengths, with the nodes still attached on each end, between two knife edge holders which engaged the nodes. A heavy leather strap placed around the centre of the internode and connected to the source of power, provided the contact for the breaking tests. Crushing was determined by placing the internodal lengths between a stationary flat iron bar and a movable iron clevice attached to the source of power. The penetration records were obtained by inserting a small punch in the movable clevice, and proceeding otherwise as in the crushing tests. The internodes were numbered in all cases from the base of the stalk upwards. Number 1 was the lowest internode free from brace roots. All results recorded are the averages of at least 10 determinations.

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Microscopic slides, for a study of the extent and distribution of lignified tissue within the stalks of the corn varieties and hybrids, were prepared in the following manner. An internodal portion was boiled in water to soften it slightly and thus avoid splintering during sectioning. Those with thin rinds were boiled about 1 minute and those with thicker rinds were boiled correspondingly longer. Transverse sections (25 microns thick) were removed from a mid-internodal region by cutting directly on a sliding microtome. An arc-shaped portion opposite to the stem groove was retained from each section; left overnight in 50% alcohol; stained for 2 hours in safranin (1% safranin in 50% alcohol); destained in acid alcohol until most of the safranin was removed from parenchyma tissue; transferred through 70% and 95% alcohol for a momentary dip in Light Green (0.2 gms. Light Green in 100 cc. of 95% alcohol); washed in 95% alcohol; dehydrated in absolute alcohol; transferred to xylol; and mounted in balsam. Four slides were prepared from the first and third internode, respectively, of each corn variety and hybrid studied.

Growing Season

The growing season in 1940, when the majority of the records were secured, was both cooler and wetter than usual. This is illustrated by Figure 1 showing the precipitation and temperature for 1940 compared with a 41-year average.

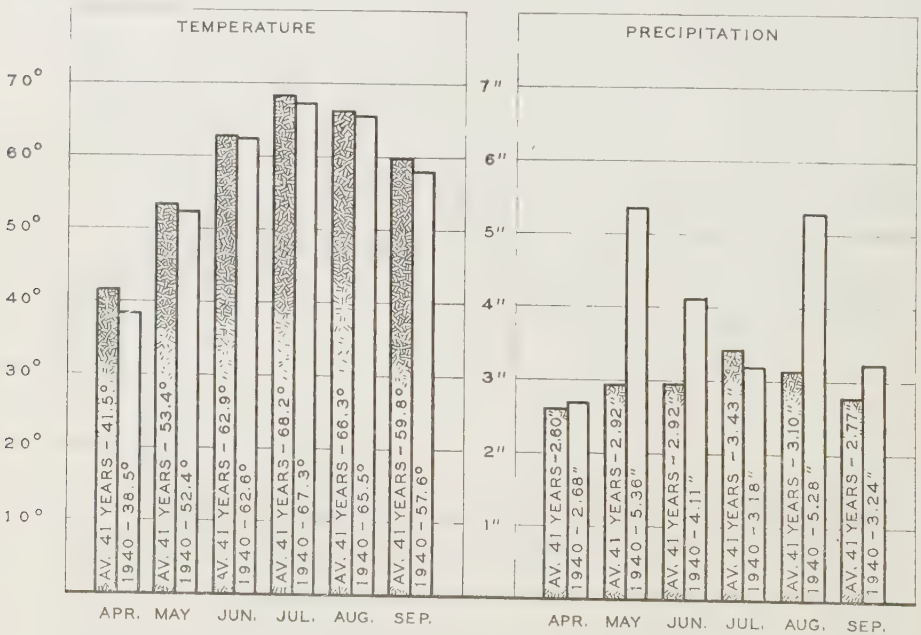


FIGURE 1. Temperature and precipitation at Guelph for six growing months, April to September, 1940, and a 41-year average.

EXPERIMENTAL RESULTS

Agronomic Data

Some agronomic records are presented in Table 1. In most instances the figures presented are the averages of 4 years. The two most common enquiries about hybrid corn are—"How does it yield?" and "How does it stand up?" A partial answer to these queries may be found in Table 1.

Ontario farmers are interested in the yields of both grain and fodder. The evidence presented indicates that, as far as fodder is concerned, all of the hybrids listed yielded substantially more than the 3 standard varieties with which they are compared. The yield of grain also compares favourably with the highest yielding of the varieties. In the case of the better adapted hybrids, the yield of grain is significantly higher.

The reported analyses of the dry fodder were made by the Department of Chemistry, Ontario Agricultural College, and indicate that as far as the percentages of protein, fibre, ash, calcium, and phosphorus are concerned the hybrids are at least equal to the varieties in feeding value. Some growers have assumed that the greater ability of the adapted hybrids to stand up under unfavourable conditions was an indication of higher fibre content, with a consequent lowering of feeding value. This contention is not borne out by the data presented.

The lodging of corn under Ontario growing conditions is due largely to inclement weather, corn borer infestations, or to a combination of both causes. Corn borer infestation records as supplied by the Entomology Department are included along with actual counts of the percentage lodging for the same years.

It is interesting to note the increasing corn borer infestation in the 3 years reported. None of the 70 different hybrids tested in 1938-39-40 showed a high degree of resistance to the attacks of this insect, but they did show a marked difference in their ability to stand up under severe infestations. In most instances the hybrids were outstandingly superior in this regard.

In 1938, two storms of unusual severity followed each other at close intervals. The corn had attained its full height, but was still quite immature at the time. A marked difference in the extent of lodging was in evidence and was decidedly in favour of the better hybrids. A fair number of these showed no signs of lodging while all varieties were badly broken over (Plate I). Stalks of several hybrids and a corresponding number of stalks of varieties were removed from the earth by washing with a hose to permit examination of the greater portion of the root structure. In all cases the hybrids showed a much more extensive root growth. This was true of both the brace roots and remaining root system (Plate I).

Data on Breaking, Crushing and Penetration

The data on breaking, crushing and penetration are presented in the form of three figures (Figures 2, 3, and 4). On each of the figures the data for the first, second, third and fourth internode are presented separately. This permits of a comparison of each internode of each lot of corn as well as of the various varieties and hybrids themselves.

Two tendencies are apparent in each of the figures. One is the rather marked decrease in resistance to breaking, crushing, and penetration in the internodes as one progresses from the lower internode upwards. This simply exemplifies the well known whip-like action of the corn stalk as a whole and is common to all lots of corn tested.

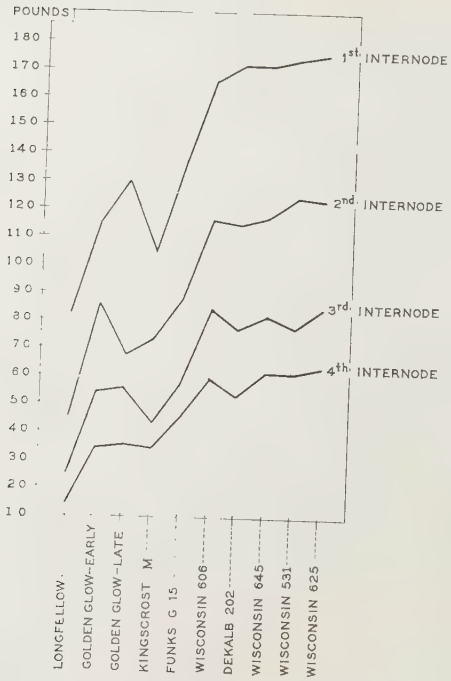


FIGURE 2. Relative resistance to breaking.

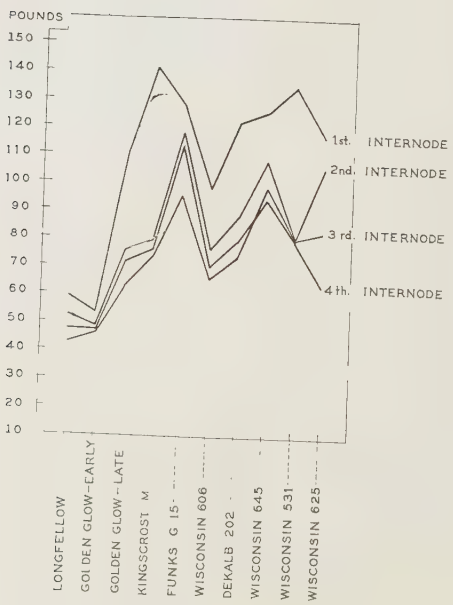
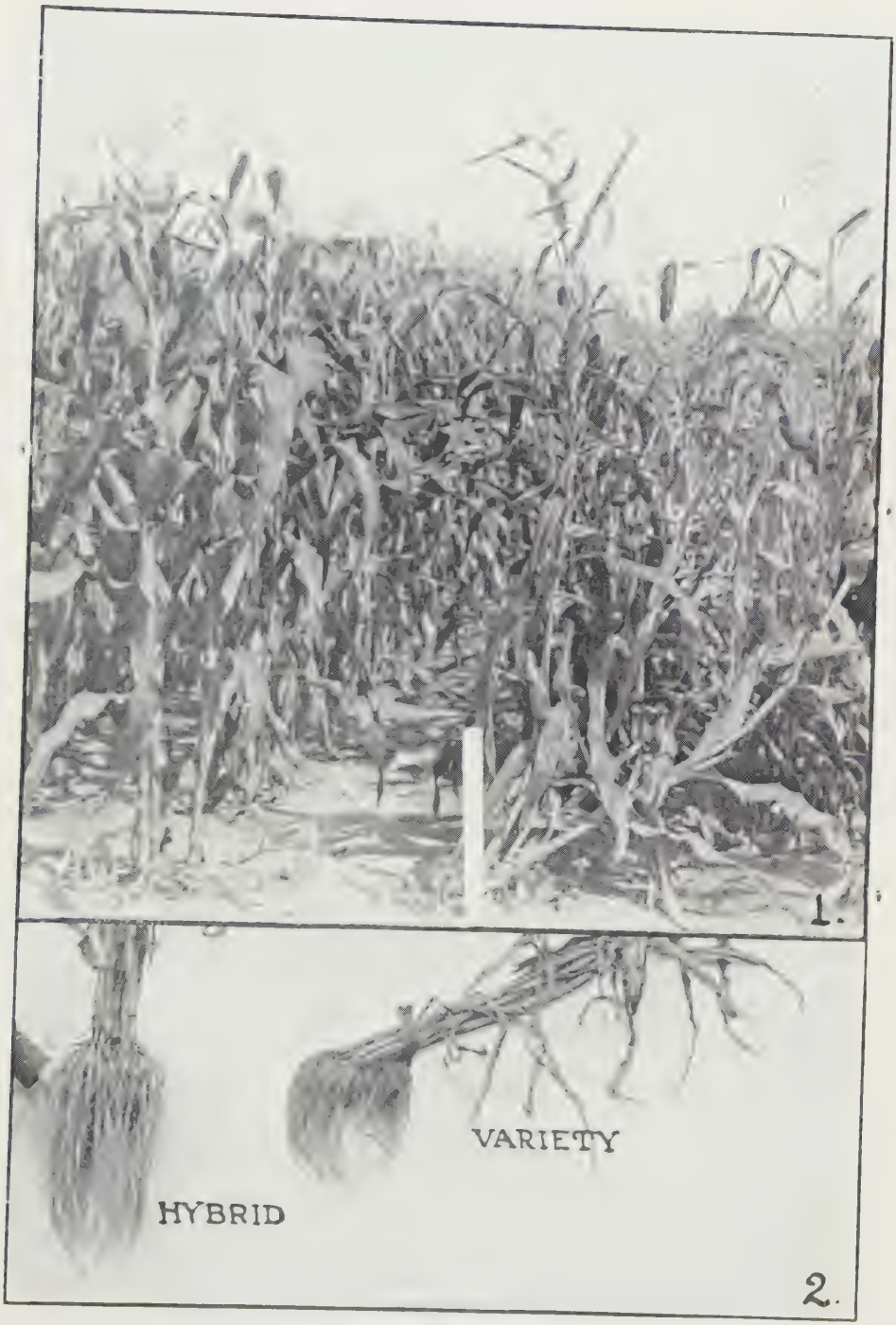
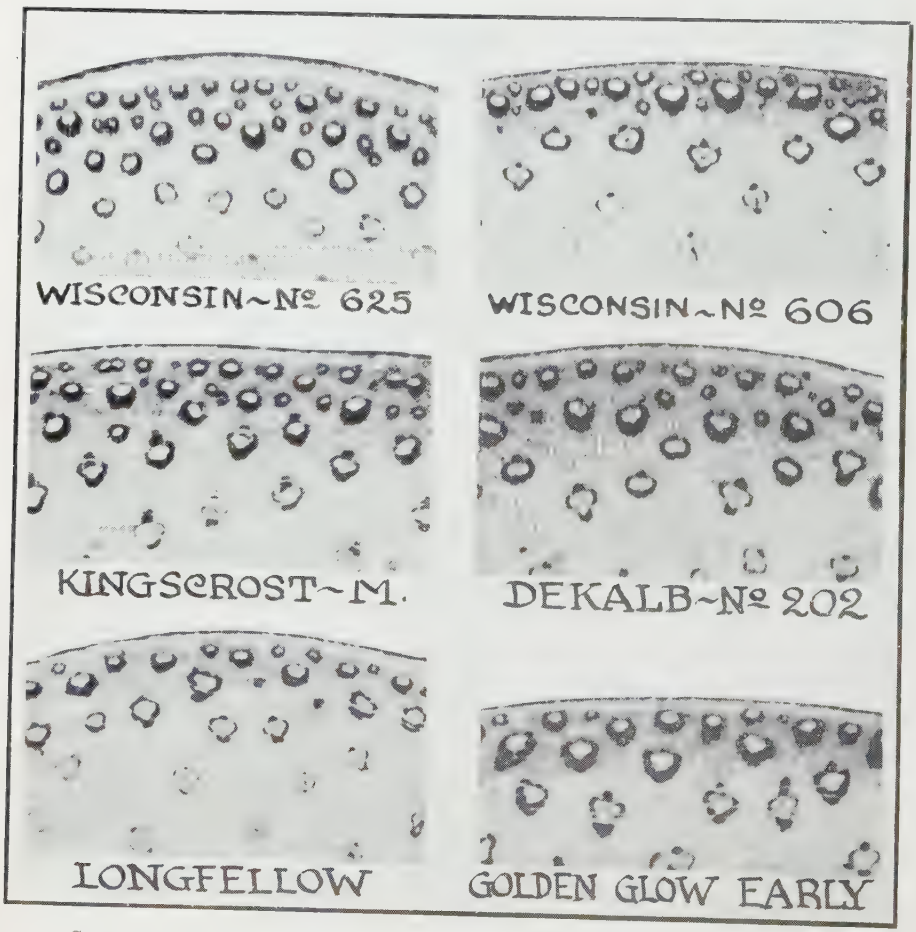


FIGURE 3. Relative resistance to crushing.



1. Visible evidence of the greater resistance to lodging of the hybrid (left) than of the variety (right).
2. Relative root development of the hybrid in comparison with the variety.



Structural variations in the extent of lignification beyond the rind and the number of vascular bundles within the lignified area in four hybrids and two varieties.

The other indication is an evident difference in the varieties and hybrids in their resistance to breaking, crushing, and penetration. On the whole, the hybrids show the greatest degree of resistance to all 3 tests. There appears to be some correlation between resistance to breaking and resistance to crushing and penetration, although this does not hold in all cases.

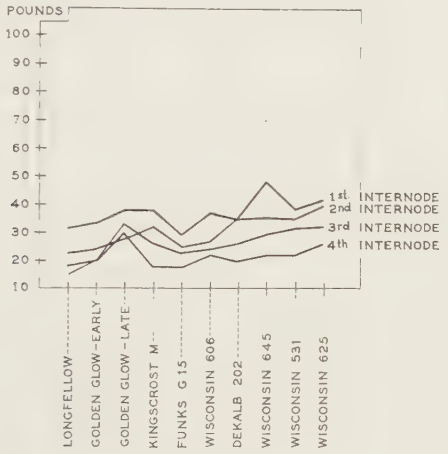


FIGURE 4. Relative resistance to penetration.

MORPHOLOGICAL STUDIES

The internal structure of the peripheral region of the first internode of the stalks of 4 hybrids and 2 varieties is illustrated in Plate II.

The significant structural variations concerned were the extent of lignification beyond the rind, and the number of vascular bundles within the lignified area. Longfellow exhibited very little lignification of the ground tissue with a correspondingly small number of vascular bundles involved; Golden Glow (early) and the hybrids, Kingscrost M, DeKalb 202, Wisconsin 625, and Wisconsin 606 exhibited a greater extent of lignified tissue and a correspondingly greater number of vascular bundles included.

While some variation occurred, the extent of lignification and number of bundles involved in the third internode were approximately three-quarters that of the first internode. According to Hershey and Martin (1) 90% of the vascular bundles are differentiated in the lower internodes of yellow dent corn by the forty-fifth day. It may be assumed, then, that all such differentiation has taken place at the time the present studies were made.

DISCUSSION

The present study deals with only a limited number of hybrids and varieties. However, the particular lots chosen are at least reasonably representative of the material being offered to Ontario growers at the present time. Undoubtedly, better adapted hybrids will be developed in the future, but the general morphological characteristics responsible for the favourable reception of hybrid corn will be, in all probability, quite similar to those of present productions.

As far as general agronomic characteristics are concerned the hybrids tested appear to have the advantage in yielding ability. The chemical analyses of the dry fodder also indicate that the hybrids are at least equal to the varieties in the constituents listed. That they are not higher in fibre is particularly interesting in view of their greater strength of stalk (the data on fibre content do not indicate the relative proportions of cellulose and ligno-cellulose). There appears to be no doubt about the adapted hybrids being definitely stronger in the stalk and that they possess a more extensive root development. Preliminary investigations indicate that the hybrids under test are as palatable as the varieties. Further studies are in progress in this connection.

The increased strength of stalk is further borne out by the tests to determine the resistance of the internodes to breaking and crushing, and to some extent their resistance to penetration as well. Of the three tests, the resistance of the internodes to crushing seems to be correlated to the greatest degree with resistance to lodging. Further tests are planned to determine the influence of the stage of maturity of any variety or hybrid on its reaction to the three tests under discussion.

A definite difference was noticeable between the cross sections of the internodal areas of the various hybrids and varieties. This was evidenced largely by variations in the extent of lignification and in the numbers of vascular bundles within the lignified area. The variation in these structural characteristics appeared to be definitely correlated with susceptibility to lodging.

Further studies are in progress regarding the morphological variations in the stalks of inbred strains, single crosses, and some top crosses. The influence of the fertility level and soil climatic zone in which the crop is grown, and the stage of maturity on the morphological structure of the internodes is also being investigated.

SUMMARY

Seven of the more commonly grown hybrids were compared with three standard varieties as to relative yield of fodder and grain, root development, strength of stalk, resistance of certain internodes to breaking, crushing and penetration, stalk structure and tolerance to corn borer.

On the whole, the hybrids were superior to the varieties in yield, resistance to lodging, and in root development.

Breaking, crushing and penetration tests on certain internodes of both hybrids and varieties furnish additional evidence of the greater strength of internode of the hybrids.

Morphological examination of cross sections of the internodes showed a greater depth of lignified tissue with a larger number of vascular bundles involved in the hybrids than in the varieties. This structural characteristic appeared to be definitely correlated with susceptibility to lodging. Further studies involving a wider range of varieties, strains and ecological conditions are in progress.

ACKNOWLEDGMENTS

The writers wish to acknowledge the generous assistance of Mr. E. G. Webb of the Agricultural Engineering Department in the development of apparatus; the Entomology Department for data on corn borer infestation; Mr. W. D. Tolton for photographs and preparation of plates; and to all other workers who assisted in compiling the general information contained in this report.

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THE DEVELOPMENT OF COBALT DEFICIENCY IN SHEEP[1]

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In a previous trial cobalt chloride was fed to ewes that had become very unthrifty after being maintained on a non-leguminous ration for a prolonged period (2). The response as measured by increase in weight and improvement in thrift was significant and suggested the probability that some of the common sheep rations in Alberta may be deficient in cobalt. This suggestion was supported by the analyses of a few feeds and by the results of cobalt research in New Zealand and the United States.

As the findings of this previous trial were based upon a relatively small number of animals whose previous nutritional regime had varied, it was deemed advisable to conduct a feeding trial with a larger number of animals maintained under carefully controlled conditions in order to obtain additional significant data.

EXPERIMENTAL PROCEDURE

Fifty ewe lambs purchased from a range flock in southern Alberta in the fall of 1938, were divided into two lots, one containing 15 ewes and the other lot 35. Both lots were fed a ration consisting of non-leguminous hays and ground oats. During most of the experiment, oat (greenfeed) hay made up one-half of the roughage allowance, the remainder being made up of timothy, brome and prairie hays fed during different periods according to which one was available at that time. Iodized salt and mono-calcium phosphate were also fed in adequate amounts.

Lot I, which was composed of the 15 ewes, also received 4 mgms. cobalt twice weekly. This was administered in the form of cobalt chloride in solution.

Lot II, comprising 35 ewes, received no cobalt.

Thus, the only difference in the ration of the two lots was in the cobalt that was fed Lot I.

Based upon the results of previous experiments, it was anticipated that the ewes in Lot II would develop symptoms of unthriftiness and loss in weight. These ewes, therefore, could be used for studying changes in the cobalt content of their tissues as well as for determining the efficacy of cobalt or other supplements in curing the condition of malnutrition.

RESULTS

Significant differences between the two lots were noted in gains in weight, reproduction, and fleece weights. These differences in the performance of the ewes were not evident until the latter part of the trial

[1] Contribution from the Department of Animal Husbandry, University of Alberta, Edmonton, with financial assistance from the Experimental Farms and the Science Services, Dominion Department of Agriculture, Ottawa, Canada.

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[3] Professor of Animal Husbandry.

[4] Professor of Animal Husbandry.

when the non-cobalt fed group failed to consume as much roughage as those receiving cobalt. It appeared that the low feed consumption which resulted in unthriftiness and malnutrition was the primary cause for the differences observed.

Gains in Weight

Both groups continued to gain equally in weight until after shearing, or 168 days after the start of the trial (see Figure 1A). At this time there was only a difference of 1·8 pounds between the average weight of the ewes in the two groups. During the period that followed, however, the difference between the average weights of the two groups increased, until by May 10, 1940, the average difference was 13·1 pounds. Based on post-lambing weights of the ewes that reproduced, the average difference was further increased to over 15 pounds between the cobalt and non-cobalt-fed ewes.

The average weights of the ewes varied to some extent with the kind and amount of feed consumed as shown in Figure 1B. Following shearing and until September 1, 1939, slough hay of fair quality was fed. This hay proved to lack palatability and the failure of the ewes to consume as much roughage as formerly resulted in many ewes losing weight. The non-cobalt-fed ewes were most seriously affected during this period.

Both groups gained at approximately the same rate during the subsequent period when fed freshly cured timothy hay, consumption being the same in both lots. Apparently the non-cobalt-fed ewes did not utilize their feed as efficiently as those receiving the cobalt. This becomes evident when consideration is given to the fact that during this period the non-cobalt ewes were from 8 to 10 pounds lighter and therefore received more hay per 100 pounds liveweight than the cobalt-fed ewes.

The critical period for the non-cobalt-fed ewes commenced just prior to the 1940 lambing period when their hay consumption declined rapidly, followed by a loss in weight, general unthriftiness, and a few deaths. Most of the ewes were removed from their lot when they had suffered serious losses in weight and had become distinctly unthrifty.

In two previous experiments in which similar basal rations were fed, unthriftiness developed following $7\frac{1}{2}$ to 9 months on the experimental ration. Mature ewes were used in the former trials and dropped lambs after approximately 6 months of experimental feeding. It was usually during the subsequent suckling period that unthriftiness developed. Ewe lambs were purchased for use in the present experiment and were not bred the first breeding season. According to the data, the non-cobalt-fed ewes showed a greater tendency to lose weight at the end of 8 months when slough hay was fed to both groups, but from this period on the non-cobalt-fed ewes remained lighter in weight than the group receiving cobalt.

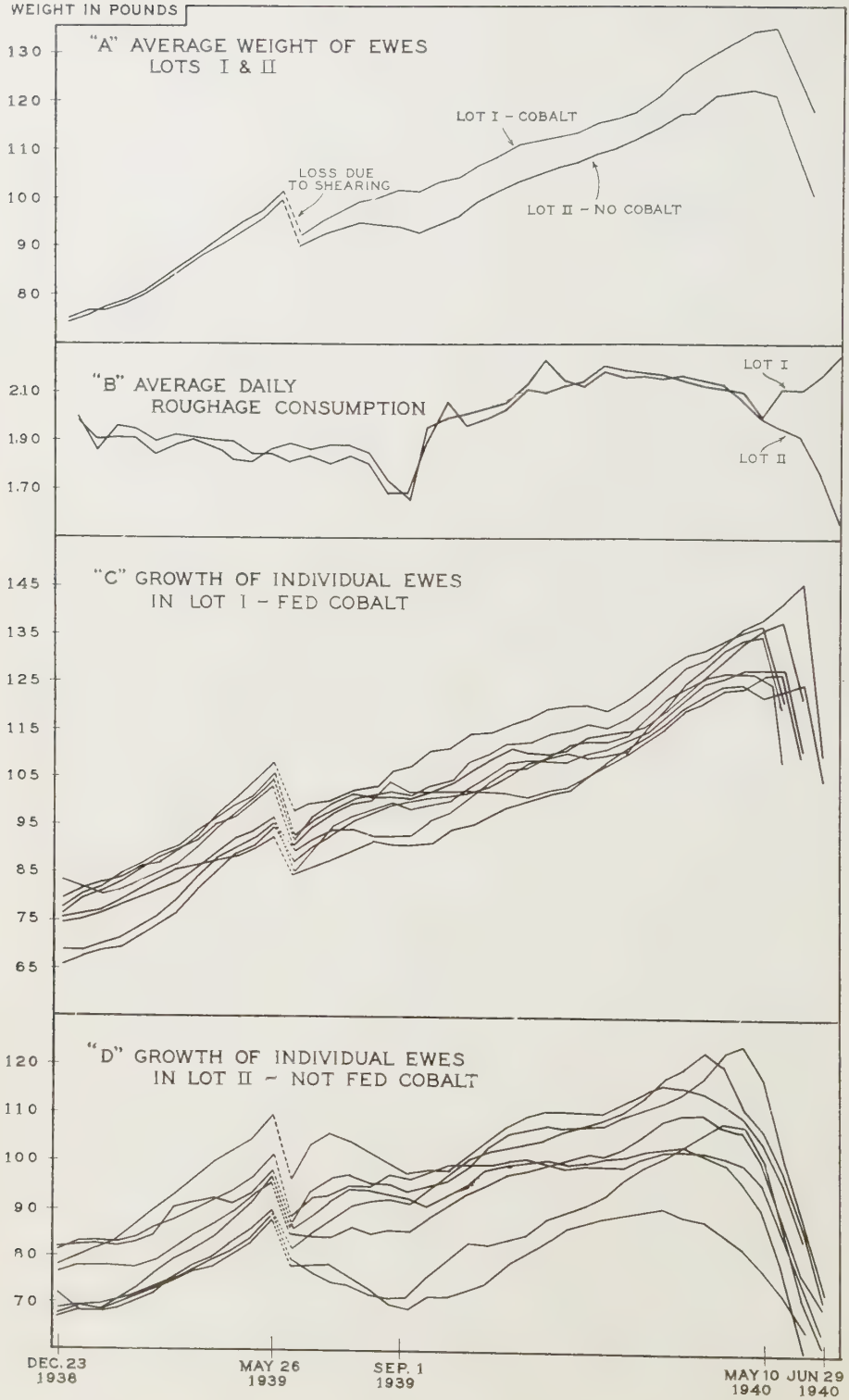

FIGURE 1. Effect of feeding cobalt on the growth and roughage consumption of sheep.

Of the 35 non-cobalt-fed ewes starting the experiment:

4 were slaughtered during the first few months of the trial
3 were removed June 3, 1940
1 died on June 6, 1940
3 were removed June 21, 1940
2 were removed June 22, 1940
1 died on June 25, 1940
5 were removed June 29, 1940
3 were removed July 6, 1940
2 were removed July 22, 1940
1 died on July 25, 1940
10 were removed August 9, 1940
—— (of these 3 were normal for flesh and thrift)
35.

The individual growth curves for 8 of the ewes that were removed in June are shown in Figure 1D. The ewes began to lose weight in March, April, and May, 1940, followed by a more rapid decline due largely to lambing and shearing. The extent of the unthriftiness of these ewes is shown by their weights after shearing and lambing. The average weight for these 2-year-old ewes was actually less than their average weights when placed on the experiment at 7 or 8 months of age. These ewes averaged 74.5 pounds at the start of the trial as lambs and at the completion of the trial 18 months later weighed an average of 70.1 pounds as 2-year-old ewes.

Figure 1C shows the individual growth curves for 8 of the cobalt-fed ewes that lambed within the same period as those in Figure 1D receiving no cobalt. It will be noted that the cobalt-fed ewes weighed between 105 and 110 pounds following lambing and shearing as compared to an average of 70.1 pounds for the 8 non-cobalt-fed ewes.

Since the cobalt-fed ewes remained heavier even though the two groups consumed equal quantities of feed, it is reasonable to assume that the feeding of cobalt must have led to a more efficient utilization of feed.

Reproduction

It is a well established fact that nutrition plays an important part in connection with reproductive performance. Such an influence was noted in the trial being reported.

Since 4 non-cobalt-fed ewes were slaughtered earlier in the trial, only 31 were in the lot at the time breeding commenced. All the 15 ewes receiving cobalt were available for breeding. The breeding period lasted from December 27, 1939, to February 8, 1940. During this time only 2 of the non-cobalt-fed ewes and 1 cobalt-fed ewe failed to come in season. Of the ewes that were bred, there were 2 non-cobalt ewes and 1 cobalt-fed ewe that failed to conceive. There was, therefore, no difference in the performance of the two groups from the standpoint of fertility. Since all the ewes in both lots appeared thrifty at the time of breeding and with a difference in average weight of approximately 7 pounds, significant difference in the occurrence of œstrum and ease of conception could not be expected.

As mentioned previously, the rapid decline in weight and loss of thrift commenced prior to and during lambing. The extent to which unthriftiness had developed and its effect on reproduction is indicated by the following lambing data.

TABLE 1.—EFFECT OF COBALT FEEDING ON REPRODUCTION

	Lot I Cobalt-fed ewes	Lot II Non-cobalt-fed ewes
No. of ewes lambing	13	27
No. of lambs dropped	13	27
Vitality of lambs at birth:		
percentage strong	92	48
percentage weak	—	41
percentage dead	8	11
Lamb mortality:		
percentage dying between:		
0– 7 days	17	58
7–28 days	—	17
28 days and date marketed	—	21
percentage marketed	83	4
Average birth weight of living lambs	9.6 lb.	7.3 lb.

These data show how seriously the lamb crop was affected. Not only were the lambs dropped by the non-cobalt ewes 2.3 pounds lighter, but these lambs were weaker, and more than half failed to live one week. Of those lambs born strong, many died because the ewes failed to provide sufficient milk to nourish their lambs.

Figure 2 shows typical ewes with their lambs from both groups. The difference in condition and general appearance is readily observed. The non-cobalt-fed ewes showed extreme gauntness, emaciation, and a listless appearance as compared to the well fleshed, rugged, and alert appearance of the cobalt-fed ewes.

The lambs from the non-cobalt-fed ewes were likewise lacking in vitality and weight, and were listless like their mothers, while the lambs from the cobalt-fed ewes were plumper and thriftier than the lambs in the other lot.

The lower birth weights of the lambs, together with their lack of vigour, suggests that the development of the nutritional deficiency symptom must have commenced some time during pregnancy. The eventual loss of all but one lamb in the non-cobalt-fed lot is an indication of how severely the lamb crop can be affected by a low cobalt intake.

Fleece Weight and Quality

Both groups of ewes were sheared in June, 1939, after they had been on experimental rations $6\frac{1}{2}$ months. The 15 cobalt-fed ewes produced a total fleece weight of 133.5 pounds for an average of 8.9 pounds. The 31 non-cobalt-fed ewes produced a total of 276 pounds of wool for the same average of 8.9 pounds. The wool from both lots graded fine staple and no difference was noted in quality.

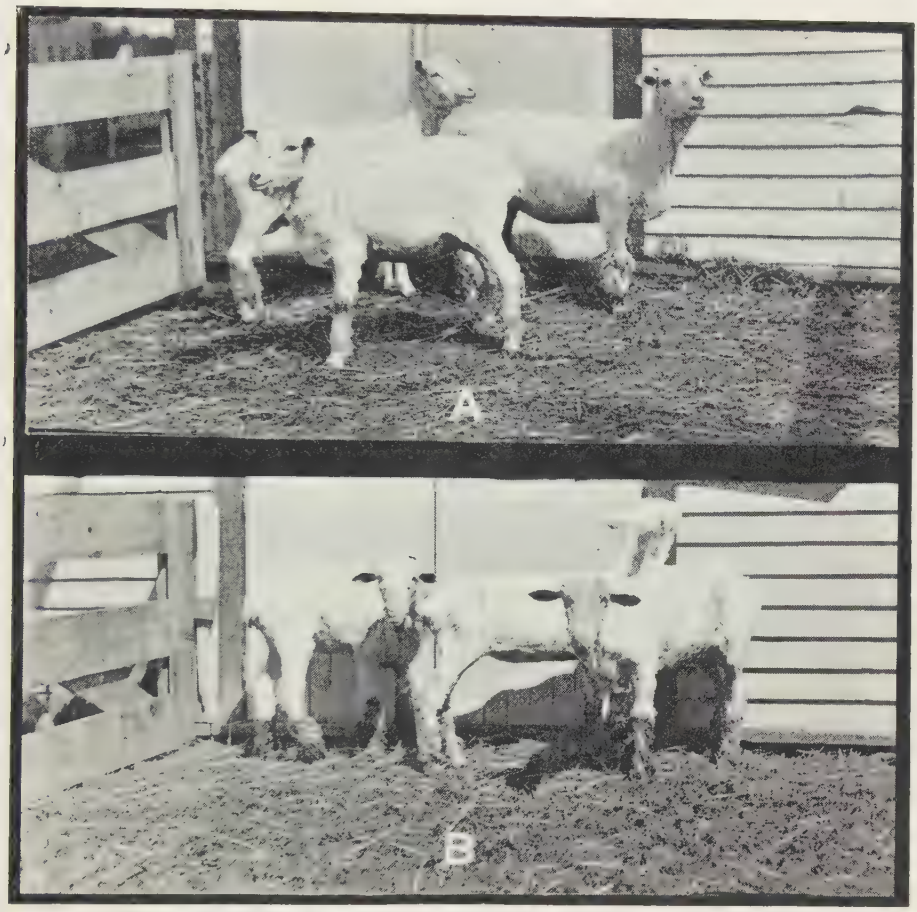

FIGURE 2A. Representative ewes of Lot I—fed cobalt.
B. Representative ewes of Lot II—not fed cobalt.

The 1940 fleeces from the two lots showed an average difference of 0·33 pounds in favour of the cobalt-fed ewes. The cobalt-fed ewes sheared an average of 9.95 pounds while the non-cobalt-fed ewes sheared an average of 9.62 pounds.

Based on the grading of the fleeces by Dominion Government graders at the warehouse of the Canadian Co-operative Wool Growers Limited at Weston, Ontario, the fleeces produced by the cobalt-fed ewes were slightly longer and stronger. The data in Table 2 show the extent of these differences.

The probable reason for so many good fleeces being secured from the non-cobalt-fed ewes was that many of them were still in a thrifty condition and the deficiency symptoms had not started to develop. Following shearing, and as additional ewes became unthrifty and emaciated, the wool fibre became weak and in some cases the wool was shed in certain parts of the body.

It would appear that the differences between the fleeces of both lots were directly caused by the differences in thrift up to shearing time and were only indirectly associated with a cobalt deficiency.

FIGURE C. Lambs from ewes fed cobalt in Lot I.
D. Lambs from ewes not fed cobalt in Lot II.
Photographs taken June 1, 1940.

TABLE 2.—EFFECT OF COBALT FEEDING ON FLEECE WEIGHT AND QUALITY

	Lot I Cobalt-fed ewes	Lot II Non-cobalt-fed ewes
No. of fleeces	15	30
Average weight of fleeces	9.95 lb.	9.62 lb.
Length of fibre:		
percentage good	20	20
percentage medium	33	17
percentage short	47	63
Strength of fibre:		
percentage strong	100	87
percentage fairly strong	—	3
percentage with weak center	—	10

Symptoms of Cobalt Deficiency

The first general symptom of cobalt deficiency that was observed was a loss of appetite and listlessness. The average daily roughage consumption as shown in Figure 1B indicates that both lots consumed equal quanti-

ties of roughage until about May 10, 1940, following which feed consumption declined rapidly in the non-cobalt-fed group. When consideration is given to the fact that during this period some of the ewes were still normal in thrift and, presumably, in their feed consumption, it follows that the roughage actually consumed by the affected ewes must have been extremely low.

In this experiment as well as in previous trials, anaemia was associated with a loss in weight. Blood determinations showed a reduction in percentage haemoglobin, red cell count, and cell volume. The average blood analyses for 5 affected ewes and 2 healthy ewes are shown in Table 3.

TABLE 3.—DIFFERENCES BETWEEN BLOODS OF AFFECTED AND HEALTHY EWES

	Affected ewes	Healthy ewes
Average haemoglobin per 100 c.c.	9.2 gm.	12.8 gm.
Average red cell count per c.c.	9.7 million	13.3 million
Averaged packed cells (vol. %)	23.8%	41.6%

There was also a reduction in blood phosphorus, and some reduction in blood protein and non-protein nitrogen, a condition that suggested improper protein synthesis. Some affected ewes developed a jaundiced condition. The above symptoms are similar to those accompanying starvation and cannot be regarded as specific for a cobalt deficiency.

Post mortem examination of the internal organs showed that these were normal with the exception of the liver. Dr. T. Lloyd Jones, Provincial Animal Pathologist, who made several post mortems of affected ewes, reported that "The whole organ was yellow-mottled on the surface and friable to the touch. When cut into, it exuded grease. Microscopically the infiltration of fat was extensive with only a few of the liver cells not being involved. The condition of the liver . . . . . is sometimes brought about by starvation."

Cobalt Content of Animal Tissues and Feed

The blood and some internal organs of a few normal and affected sheep were analysed for cobalt with the object of determining to what extent the cobalt in certain tissues had become depleted. Table 4 shows the results of these analyses together with comparable figures published by Askew and Dixon (1) and Underwood and Harvey (5).

According to the analyses the concentration of cobalt in normal blood was low, and therefore the depletion could not be great. There was a much higher concentration of cobalt in the liver and spleen of the thrifty non-cobalt ewes than in the blood. This higher cobalt level, however, was reduced to an extremely low level in the unthrifty ewes.

While only a few cobalt analyses of organs were made, the data secured were somewhat similar to those reported by the research workers in Australia and New Zealand. The data do suggest that cobalt depletion of the tissues of the non-cobalt-fed ewes did accompany the deficiency symptoms.

TABLE 4.—EFFECT OF COBALT FEEDING ON THE COBALT CONTENT OF BLOOD AND ORGANS WITH SIMILAR DATA FROM NEW ZEALAND AND AUSTRALIA

	Lot I Cobalt-fed-ewes		Lot II Non-cobalt-fed-ewes			
			Thrifty		Unthrifty	
	No. of ewes	Cobalt p.p.m.	No. of ewes	Cobalt p.p.m.	No. of ewes	Cobalt p.p.m.
Blood:						
after 8 months on experiment	2	0.04	2	0.02	2	0.00
after 14 months on experiment	2	0.02	2	0.01	2	0.01
after 19 months on experiment	2	0.02	—	—	8	0.01
Liver:						
after 5 months on experiment	—	—	4	0.18	—	—
after 19 months on experiment	—	—	—	—	4	0.02
Spleen:						
after 5 months on experiment	—	—	4	0.18	—	—
after 19 months on experiment	—	—	—	—	4	0.02
Kidney:						
after 19 months on experiment	—	—	—	—	4	0.02
Results of Askew, New Zealand:						
Blood cobalt	2	0.045	8	0.02	2	0.02
Liver cobalt	2	0.225	8	0.12	2	0.02
Results of Dixon, New Zealand:						
Blood cobalt	3	0.03	—	—	3	0.01
Liver cobalt	4	0.2	—	—	6	0.025
Results of Underwood, Australia:						
Liver cobalt	5	0.26	—	—	10	0.06

The feeds fed were also analysed. The hays were secured from several sources.

2 samples of oat hay fed contained an average of 0.02 p.p.m. cobalt.

5 samples of timothy hay fed contained an average of 0.01 p.p.m. cobalt.

2 samples of oat chop fed contained an average of 0.02 p.p.m. cobalt.

1 sample of slough hay fed contained an average of 0.015 p.p.m. cobalt.

Samples of oat and timothy hays secured from various parts of the province showed relatively small amounts of cobalt.

18 samples of oat hay in 1939 contained an average of 0.02 p.p.m. cobalt, while

6 samples in 1940 averaged 0.01 p.p.m. cobalt.

8 samples of timothy hay in 1939 contained an average of 0.03 p.p.m. cobalt, while

5 samples in 1940 averaged 0.03 p.p.m. cobalt.

The cobalt content of the soils and pasturage in districts of Australia and New Zealand where "bush sickness" was prevalent was decidedly

lower than the soils and pasturage of "healthy" areas. In "pining" areas in Scotland the cobalt content of herbage was likewise found to be low (3). According to McNaught (4) "pastures from typically 'bush-sick' country contained, on the average, less than 0.04 p.p.m. cobalt, whereas pastures from healthy areas averaged nearly 0.11 p.p.m. cobalt."

In the trials being reported the cobalt content of the feed was below that of the average pasturage from the unhealthy districts of both Australia and New Zealand.

Response of Unthrifty Ewes to Cobalt

While it would appear that the differences between the performance of the two groups of ewes were caused almost wholly by differences in their cobalt intake, additional data were secured to determine whether or not the unthrifty ewes would regain their normal condition of health by the feeding of cobalt.

As previously mentioned, of the 31 original ewes in Lot II not fed cobalt, 28 developed deficiency symptoms. Of these, 8 were selected for the purpose of studying their response to the feeding of cobalt at the same rate as was being fed to the ewes in Lot I.

The rapid gains made by these unthrifty ewes following the feeding of cobalt is shown in the following table.

TABLE 5.—EFFECT OF COBALT FEEDING ON EWES AFTER DEFICIENCY SYMPTOMS HAD DEVELOPED

—	Lot I Cobalt-fed ewes	Lot IIA Not fed cobalt prior to development of unthriftiness	Difference in weight
No. of ewes	14	8	—
Average weight of ewes:			
when cobalt feeding started in Lot IIA	106.4	70.8	35.6
After 4 weeks	106.2	85.8	20.4
After 8 weeks	104.7	90.2	14.5
After 16 weeks	109.9	99.0	10.9
After 8 to 9 months	134.6	124.5	10.4

It would appear from these data that the growth of the ewes had been permanently affected.

Individual weight curves for the 8 ewes of Lot II that developed deficiency symptoms and were subsequently fed cobalt, and 8 typical ewes from Lot I fed cobalt throughout the experiment, are shown in Figures 3A and B. Figure 3B shows clearly the rapid gains in weight resulting from cobalt feeding, whereas Figure 3A shows a very slow gain made by the original cobalt-fed ewes of Lot I.

These data give added proof that the unthriftiness of the non-cobalt-fed ewes was due to a lack of cobalt in the ration.

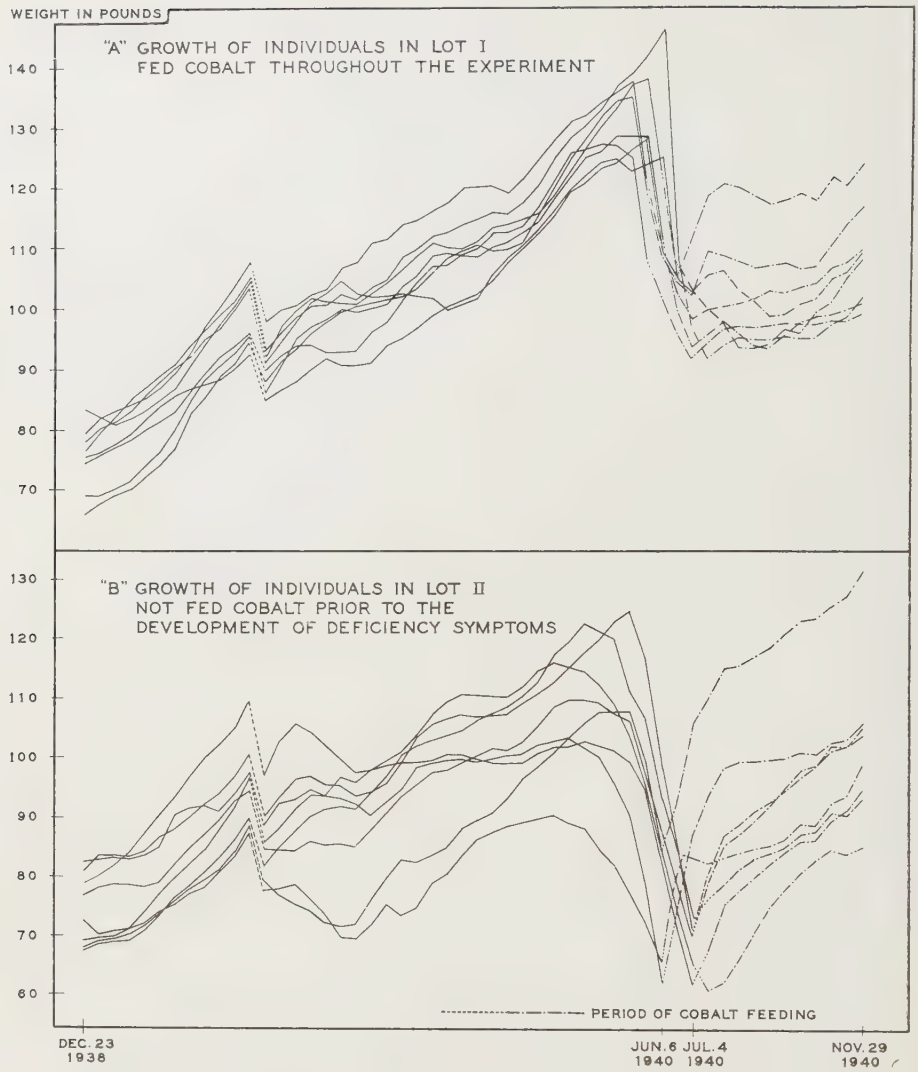

FIGURE 3. Effect of feeding cobalt to ewes after development of cobalt deficiency symptoms.

SUMMARY

When sheep were maintained for a relatively long period on a dry ration of non-leguminous hays, oats, and certain mineral supplements, a nutritional deficiency developed. The addition of cobalt to such a ration prevented the development of deficiency symptoms and brought about recovery in the case of affected sheep.

The clinical symptoms of sheep fed on cobalt-deficient rations appeared to be similar to those of malnutrition. Reproduction was seriously impaired in that lambs from affected ewes were smaller and weaker than lambs from unaffected ewes. Ewes that became unthrifty on cobalt-deficient rations produced insufficient milk to nourish their lambs, and also produced fleeces that were weak in fibre.

With the development of the deficiency symptoms there was a decrease in the cobalt content of certain tissues. This corresponds with results reported from areas elsewhere known to be deficient in cobalt.

The cobalt content of the feeds comprising the ration averaged much lower than pasturage grown in "bush-sick" areas of Australia and New Zealand.

Even though the feeds utilized in the experiment were among those commonly fed to sheep in Alberta, and while the occasional occurrence of symptoms similar to those which developed in the experiment have been reported from sheep raising areas, more data would have to be secured before it could be established that cobalt deficiency constitutes a limiting factor in economical sheep production in the Province.

ACKNOWLEDGMENTS

The authors desire to express to Dr. G. Hunter, Department of Biochemistry, University of Alberta, their appreciation of his valued suggestions offered from time to time during the progress of this research. Analysis of internal organs and feed was conducted by the Industrial Laboratory, University of Alberta.

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THE FEEDING VALUE OF CANADIAN WESTERN GRAINS FOR BACON HOGS[1]

I. THE EFFECTS OF WEIGHT PER BUSHEL AND OF SAMPLE PURITY ON THE FEEDING VALUE OF BARLEY

II. THE RELATIVE FEEDING VALUE OF CANADIAN WESTERN BARLEY, DURUM WHEAT, FEED WHEAT AND No. 1 RECLEANED SCREENINGS

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Canadian Western feed barley is graded into three qualities which are distinguished largely by weight per bushel and purity of sample. Feeding tests (1) have shown that the feeding value decreases with decreasing quality of feed, but the design of these previous trials did not permit separate measurement of the importance of weight per bushel as compared to proportion of dockage present in causing the differences in feeding value.

In addition there was the question of the value of wheat and wheat screenings as compared to the coarse grains. This was of special interest because of the possibility that Durum wheat might be raised for feeding where neither milling wheat nor barley were suitable crops.

In order to obtain information on these matters a hog feeding trial was carried out at Macdonald College during 1940. The test was in effect a continuation of the co-operative barley feeding projects started in 1938 sponsored by the National Barley Committee.

EXPERIMENTAL PROCEDURE

The design of the test was similar to that of the previous studies in this series excepting that it was possible to use 10 pigs per lot and to arrange for equal numbers of males and females in each lot. The essential features of the plan of procedure followed in all tests of this series are abstracted below:—

Animals: Bacon type pigs, preferably of Yorkshire breeding, put on test rations at an average age of 70 days and weighing at that age not less than 35 pounds each.

Allotment: All feeding lots to consist of 10 pigs, allotted with due consideration to age, weight, sex, condition and breeding. In this trial all pigs will be housed and fed individually throughout the test.

Feeding Periods: In general, the feeding periods will correspond to those followed at the Advanced Registry Pig Testing Stations. This calls for pigs to be fed a growing ration up to a weight of 100 to 110 pounds, after which they are changed to a finishing ration. Each pig will be shipped for slaughter on reaching a weight of 200 pounds.

Feeding Practice: Until pigs reach 100–110 pounds, they are to be fed three times daily, and twice thereafter. All pigs to be hand fed to the limit of their appetites. The dry meal allowance at each feeding is to be measured for each pig and the water allowance to be added at the time of feeding. During the first period

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 162.

[2] Associate Professor of Animal Nutrition, Macdonald College (McGill University), Quebec.

(to 100–110 pounds) all pigs are to receive 15 cc. of "Noppco XX" cod liver oil daily. This oil carries a guaranteed potency of 3,000 I.U. vitamin A and 400 I.U. of D. Pigs are to be penned and confined indoors throughout the test.

Feed Mixtures and Rations Used: The grain comparisons involved in the test herein reported are shown in Table 1.

TABLE 1.—GRAINS USED IN 1940 HOG FEEDING TEST

Lot	Grain	Description as to quality
I	No. 1 feed barley	A sample typical of the official grade.
II	No. 3 feed barley	A sample carrying the usual quantities of dockage, and with the barley itself of light weight per bushel.
III	Recleaned barley adulterated with maximum tolerance of wild oats and seeds	A quantity of the No. 1 feed barley used in Lot I was recleaned to obtain a pure barley of standard weight per bushel. To this was added the amounts of wild oats (17%) and weed seeds (3%) tolerated in No. 3 feed barley.
IV	No. 2 C.W. amber Durum wheat	Typical sample.
V	Feed wheat	Typical sample having thin shrunken kernels.
VI	No. 1 recleaned wheat screenings.	Typical sample, largely broken wheat kernels and wild buckwheat.

The ration combinations used are given in Table 2.

TABLE 2.—COMPOSITION OF MIXTURES USED*

—	I	II	III	IV	V	VI
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
No. 1 feed barley	85					
Recleaned barley			68			
No. 3 feed barley		85				
Wild oats			14.5			
Seeds			2.5			
Durum wheat				85		
Feed wheat					85	
No. 1 recleaned screenings						85
Protein-mineral supplement	15	15	15	15	15	15
	%	%	%	%	%	%
Chemical analysis of mixtures—						
Moisture	11.86	11.42	11.47	11.00	11.47	11.57
Crude protein	17.21	17.40	16.26	20.56	20.28	18.76
Crude fat	1.97	2.41	2.82	1.46	2.68	3.18
Crude fibre	4.99	5.60	6.57	2.67	5.55	4.97
N-free extract	57.71	56.82	56.31	58.62	54.20	55.67
Ash	6.26	6.35	6.57	5.69	5.82	5.85

*For pigs from weaning to 100 lbs. For pigs from 100 lbs. to market weight, the protein-mineral supplement was reduced to 10 lbs. in 100 lbs. of mixtures, and basal feeds increased proportionately.

The protein-mineral supplement (see Table 2) consisted of:—

50% Tankage (60% protein)
20% Linseed oilmeal (39% protein)
15% Fishmeal (non-oily)
5% Fine salt (iodized 1..6 oz. per 100 lbs.)
5% Ground limestone
5% Feeding bone meal.

STATISTICAL ANALYSIS OF THE DATA

The design of the experiment was such that it was possible to analyse the variance for each of the items of data recorded into the fractions indicated in Table 3.

TABLE 3.—ANALYSIS OF VARIANCE OF INITIAL WEIGHT

Variance due to	D/F	Mean square (σ^2)	Standard deviation	*F* values	
				Observed	Necessary
All causes	59				
Between six lots	5	8.00		—	2.41
Between two sexes	1	.60		—	4.04
Interaction sex × lot	5	5.88		—	2.41
Residual	48	8.38	2.89		—

Necessary difference between lots to cover uncontrolled variation $= \frac{2.89}{\sqrt{10}} \times \sqrt{2} \times 2.01 = 2.60$ lbs.

TABLE 4.—MEANS AND STANDARD DEVIATIONS OF ITEMS RECORDED IN FEEDING TEST

Item	Mean	σ	Coefficient of variability %	*F* values*	
				Between lots	Between sexes
Initial age (days)	69	5.4	8	2.8	—
Initial weight (lbs.)	45	2.9	6	—	—
Daily feed (lbs.)	5.9	.34	6	3.0	—
Daily gain (observed) (lbs.)	1.7	.13	9	4.1	6.3
Feeding period (days)	91	7.4	8	5.1	15.0
Age at shipping (days)	159	9.0	6	1.9	8.8
Shipping weight (lbs.)	200	9.0	4	—	2.2
Carcass score (%)	76	11.4	15	—	4.7
Carcass weight (lbs.)	147	8.0	5	1.6	4.8
Carcass length (in.)	30.1	1.0	3	—	2.8
Percentage shoulder (%)	28	1.7	6	1.3	1.7
Percentage middle (%)	47	1.5	3	4.3	13.3
Percentage ham (%)	24	.9	4	3.6	13.7
Shoulder fat (in.)	1.6	.24	15	—	5.9
Back fat (in.)	1.1	.13	12	2.6	11.9
Fat firmness (thumb)	1.4	1.07	75	—	3.7
Fat evenness (in.)	.5	.18	33	—	—
Max. belly thickness (in.)	1.5	.19	12	—	—
Min. belly thickness (in.)	.8	.10	13	6.0	10.5
Area of loin muscle (sq. in.)	5	.6	13	1.5	21.6
Percentage lean in rasher (%)	47	4.5	9	5.2	21.7

*F values necessary for significance at P = .05—between lots, 2.41; between sexes, 4.04.

RESULTS

Table 4 gives the general means and standard deviations for the data recorded together with the F ratios of variances between lots and between sexes to the residual (error) variance. Where the residual variance is larger (i.e. when the ratio is less than 1), no values are shown, there being no question of the insignificance of the lot or sex grouping.

The figures in Table 4 bring to light a factor in bacon hog production which heretofore has frequently been disregarded—namely that of sex. Out of the 18 items recorded which were not controlled by the design of the test (initial weight and age, and shipping weight were controlled by design) the sexes differed significantly in 12, while ration groups differed in but 8 of these items.

Before turning to Table 5 where these differences are brought out more in detail, it may be well to note the average results obtained in this test as a whole. The average rate of gain from the initial weight of 45 lbs. per pig to market weight of 200 lbs. was 1.7 lbs. per day. The feeding period necessary was 91 days, and the pigs were shipped to market in just less than 160 days of age. The carcasses scored on the average 76 out of a possible 100 points; graded firm; and carried about 47% of lean in the rasher. It seems evident that the rapid gains obtained by full feeding are not incompatible with desirable carcass characteristics. Furthermore, the absence of milk need be no handicap to pig raising from the standpoint of preparation of a suitable ration.

TABLE 5.—SUMMARY OF RESULTS SHOWING AVERAGE GROUP VALUES

Items	Feed groups							Sex groups		
	Lot I	Lot II	Lot III	Lot IV	Lot V	Lot VI				
	No. 1 barley	No. 3 barley	Pure barley plus dockage	Durum wheat	Feed wheat	Wheat screenings	Nec.* diff.	Male	Female	Nec.* diff.
Initial age (days)	72	64	67	68	71	69	5	69	68	3
Initial weight (lbs.)	46	45	44	45	45	45	3	45	45	1
Daily feed (lbs.)	6.1	5.6	5.8	5.9	6.0	6.1	0.3	6.0	5.9	0.2
Daily gain (observed) (lbs.)	1.74	1.56	1.65	1.82	1.75	1.80	0.14	1.77	1.67	0.08
Daily gain (adjusted) (lbs.)	1.70	1.62	1.68	1.82	1.74	1.77	0.12	1.76	1.68	0.07
Feeding period (days)	88	99	95	85	89	87	7	87	94	4
Age at shipping (days)	161	163	162	153	160	156	8	156	163	5
Weight at shipping (lbs.)	200	198	200	199	200	201	8	198	201	5
Carcass score (%)	73	77	77	70	78	79	10	72	79	6
Carcass weight (lbs.)	149	146	148	149	141	148	7	144	149	4
Carcass length (in.)	30.3	29.9	29.8	29.9	30.2	30.6	0.9	29.9	30.3	0.5
Percentage shoulders (%)	28.5	29.5	28.4	27.8	28.3	27.8	1.5	28.1	28.7	0.9
Percentage middle (%)	47.5	46.0	47.2	49.1	47.4	47.6	1.4	48.2	46.7	0.8
Percentage hams (%)	24.0	24.5	24.4	23.1	24.3	24.5	0.8	23.7	24.6	0.5
Shoulder fat (in.)	1.7	1.6	1.6	1.7	1.6	1.6	0.2	1.7	1.5	0.1
Min. back fat (in.)	1.1	1.0	1.0	1.2	1.0	1.0	0.1	1.1	1.0	0.1
Min. belly thickness (in.)	.8	.8	.7	.9	.7	.8	0.1	.8	.7	0.05
Max. belly thickness (in.)	1.6	1.5	1.5	1.6	1.5	1.5	0.2	1.5	1.5	0.1
Area eye of lean (sq. in.)	5.1	5.1	5.5	4.8	5.4	5.0	0.6	4.8	5.5	0.3
Percentage lean in rasher (%)	47	48	50	41	50	47	4	44	50	2

*P = 0.05.

In Table 5 will be found the averages for each lot. Values for the male and the female pigs are also included to show more clearly the marked sex differences found in several of the items. The averages for sex are not given for each lot but are grouped for each item. This was permissible since there was no interaction between sex and ration found in any case, the trend of sex differences being the same in each ration group.

EFFECTS OF SEX

Considering the sex differences first, it seems evident that rate of gain (whole feeding period) is likely to be about 0.1 lbs. faster with male pigs than with females. This means a reduction in feeding period of about a week and since correspondingly higher feed intake was not found, indicates a slight increase in ration economy with males.

There have been effects of sex on carcass also. Male carcasses were slightly shorter and considerably fatter. Neck fat, belly thickness, percentage middle, and percentage lean in rasher all reflect this condition.

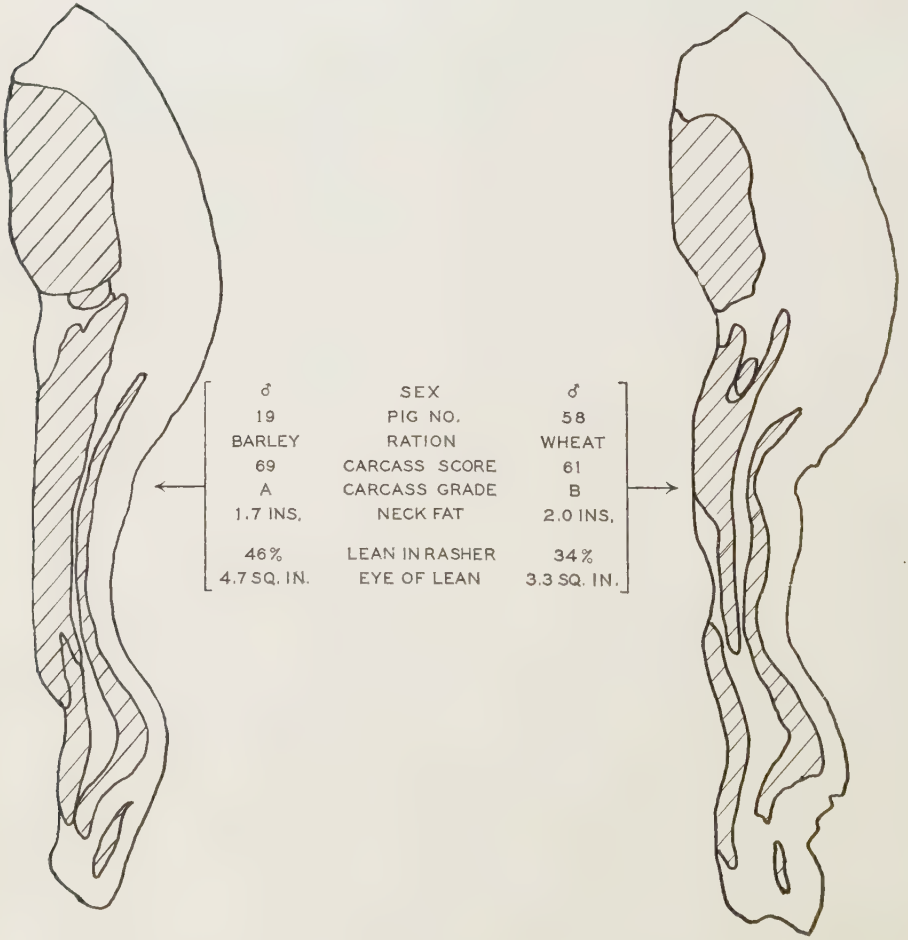

FIGURE 1. Typical rashers—male pigs.

The male carcasses were very slightly firmer than the females, but neither were penalized in this respect. However, largely because of higher grading bellies (not shown in tables here) and a larger "eye of lean" the female carcasses obtained a significantly higher average score for general excellence.

RATION COMPARISONS

In so far as data from the live hog are concerned, the items of greatest interest and significance are those of rate of gain and of feed consumption. Comparisons between Lots II and III are in effect comparisons of light weight vs. standard barley; while Lots I and III compare the effect of dilution of heavy barley with wild oats and weed seeds. Differences between Lots I and II are the result of the combination of poor barley and dockage.

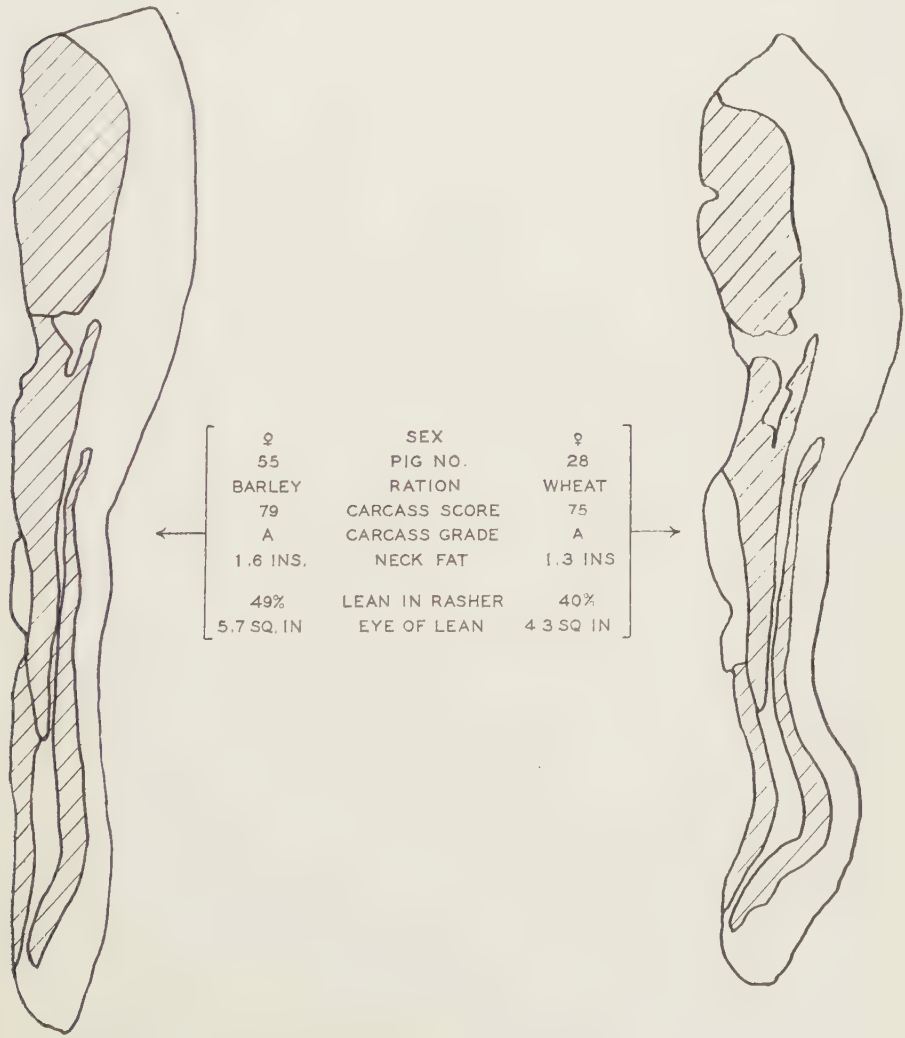

FIGURE 2. Typical rashers—female pigs.

From the start, the pigs in Lot I led those of Lots II and III in gains. At market weight these three lots ranked:—

Lot I—(Heavy barley + 4% dockage)—1.74 lbs. per day.
Lot III—(Heavy barley + 20% dockage)—1.65 lbs. per day.
Lot II—(Light barley + 20% dockage)—1.56 lbs. per day.

From this showing it would appear that poor quality barley and the tolerance of dockage permitted in the No. 3 feed grade have about equal effects in depressing the rate of live weight gains, and that these effects are additive.

The explanation for these results in gain is found largely in the feed intake records, with respect to which these three lots ranked in the same order as in live weight gain. If the gain figures are adjusted (by regression) to the general average feed intake, most of the difference between the lots disappears. Actually the daily gains adjusted to equal feed intake are 1.70; 1.68; and 1.62 for Lots I, III and II respectively. Evidently both the inclusion of 20% dockage and the use of light weight barley decrease the acceptability to pigs of the meal over that from No. 1 feed barley.

The wheat fed lots have stood above the barley groups both in absolute rate of gain and in gains adjusted to equal feed intake. Between the three wheat fed lots, Durum wheat (Lot IV) ranked slightly above the wheat screenings fed group. Feed wheat, which consisted of thin shrunken kernels, stood in third place. Feed intake in these groups did not follow the same order as the gains. Indeed there were no marked differences between the lots in this respect. Screenings were evidently very palatable, and all wheat rations were eaten in slightly greater amounts than the barley mixtures of Lots II and III. It might be argued that between Lots IV, V, and VI the lower value of feed wheat (Lot V) (as in the case of No. 3 feed barley) was traceable to a lower quality of grain (i.e. light weight per bushel), or to the inclusion of some dockage.

What differences there were in the carcass measurements traceable to ration were related chiefly to degree of finish. For example, the Durum wheat lot carcasses were fatter. This condition not only affects depth of shoulder and back fat, but because of the relative surface areas of the cuts, results in a higher percentage of the carcass weight which will be in "middle." Correspondingly the percentage ham and/or shoulder will be reduced.

The area of "eye of lean" in the bacon rasher was least in the Durum wheat lot, and it is interesting to note that this is similar to the effects found in previous tests with heavy corn feeding. While the Durum wheat gave results somewhat similar to those from corn feeding in so far as degree of finish was concerned, wheat did not tend to soften the carcass. The reduction in percentage of lean in the rasher in Lot IV is also very apparent.

Carcass score is an "over all" index of carcass excellence. The particular scheme by which carcasses were marked in this test is that used by the Advanced Registry for Swine (see Sci. Agri. 20 : 7, 1940) and involves weight, length, degree of finish, balance of side, size of eye of lean and belly grade. All carcasses in this test were scored by the Dominion hog graders on duty at the packing plant when the hogs were marketed. A comparison of the ration groups in respect to carcass score is interesting.

It will be seen that the Durum wheat fed carcasses stood lowest with a score of 70 and that other groups excepting No. 1 barley ranged between 77 and 79 out of a possible 100 points. The No. 1 barley group scored 73. In studying the make up of the scores it appears that the largest factor in the low scores in the Durum wheat lot was the small eye of lean in the bacon rasher. These carcasses were below average also in grade of belly. The No. 1 barley group also lost points, but to a lesser extent, for belly grade and for balance of side. Because of the composite nature of the score it is difficult to relate it to any special characteristic in the individual carcass unless it is one of marked and consistent occurrence.

It may be argued that the "rail grade" is the more practical index of carcass excellence than carcass score. Certainly it is the index on which the feeder does or does not receive the bonus award. The differences associated with the feeding groups in this respect are interesting.

It will be noted that while 8 of the carcasses from hogs fed No. 1 barley received the bonus, only 3 from the Durum wheat lot made the A grade. B grade carcasses from Durum wheat feeding were chiefly penalized (rail grade) because of over fatness while those of the feed wheat group were penalized for lack of finish. Taking all the barley fed hogs, 70% graded A as compared to 44% from the wheat feeding. It is interesting to note that the A grade carcasses averaged 80 in carcass score as compared to 71 for B grade carcasses.

TABLE 6.—RAIL GRADES* OF CARCASSES

—	No. 1 barley	No. 3 barley	Pure barley plus dockage	Durum wheat	Feed wheat	Wheat screenings
Male	B_1	B_1	A	B_1	A	A
	A	A	B_1	A	A	A
	A	A	A	B_1	B_1	B_1
	A	A	A	A	A	B_1
	B_1	A	A	B_1	B_1	A
Female	A	B_1	B_1	B_1	A	B_1
	A	A	A	B_1	B_1	A
	A	B_1	A	B_1	B_1	A
	A	B_2	B_1	B_1	B_1	A
	A	A	A	A	B_1	B_1

*A bonus is paid for each grade A carcass.

In an attempt to depict the trends of difference between carcasses from the barley as compared to the wheat rations and between sexes, the tracings of 4 rashers are shown in Figures 1 and 2. From a comparison of these example rashers the sex differences in size of "eye of lean" are clearly evident. Females tend to carry less back fat and correspondingly a greater percentage of lean. The extra thickness of belly of the males (presumably due to increased fat) has resulted in lower grades for belly.

Another difference which has been found in these carcasses is the presence at the flank end of the rasher of each male pig of a small area

of lean not found in any female rasher. This is of some interest in view of intimation that breakfast bacon showing a streak of lean in this location is preferred by the housewife to slices that have a clear fat area at the flank end. Whether or not this is a sex-linked hereditary characteristic is not known, but it accurately differentiates male and female carcasses in this group of 60 hogs.

Ration differences are also clearly seen from the tracings in Figure 1. Wheat rations show the tendency to a heavy fat layer on the side of the rasher which was also found in corn fed carcasses. The excessive fatness from wheat was more pronounced in male than female carcasses, however.

DISCUSSION

The ration differences found in this test, especially when taken in conjunction with the results of the previous trials in this series, lead to the suggestion that there may be some factor present in corn and in high grade wheat which acts to stimulate fat synthesis or fat deposit in body depots. This factor must be absent, present in lesser amounts or inactive, in barley, damaged wheat, screenings, and oats. In other feeding trials at this station in which a ration of No. 1 feed barley fortified with meat meal, fish meal, oilmeal, yeast and minerals has been used, addition of 3% of defatted wheat germ has resulted in an increase in feed intake and in rate of gain. The tendency toward the heavy fat deposit on the side of the rasher was not noticeable, however. McHenry *et al.* (2, 3, 4, 5) has shown with rats that several fractions of the vitamin B complex (known to be present in wheat germ) are closely associated with the synthesis and deposition of fat either from protein or from carbohydrate.

The question arises as to whether immaturity or damage such as freezing destroys or alters the effectiveness of the "fat stimulating" factor and whether or not corn and wheat germ are peculiar in respect to source of such a factor. Common experience with oat feeding has led to the opinion that this grain is not a highly fattening feed. Carcasses of oat-fed pigs in the first tests of this series were penalized for lack of fat at the same weight of pigs. This has usually been interpreted as the result of the higher crude fibre content of the oats. In the tests herein reported, carcasses from Durum wheat when faulted on the rail grading were penalized for over finish. Contrarily, penalties awarded the carcasses from the feed wheat feeding were because of lack of finish.

The differences between barley-fed, and either corn- or wheat-fed carcasses can hardly be accounted for on the basis of crude fibre (as has often been the case with oats) because actual rates of gain have been as high or higher on the barley rations (as on corn), and there is no basis for believing that the presence of crude fibre has any tendency to stimulate production of lean tissue, nor its absence to depress it.

McHenry's work indicates that specific substances extractable from liver and from pancreas are also involved with fractions of vitamin B in fat synthesis and deposition. The differences between the sexes in their tendency to fatten could be ascribed to hereditary differences in endocrine balance and hence to the abundance or nature of McHenry's liver extract of the pancreatic "lipocaic". The differences in the ability of the rations

in this test to stimulate fat production cannot be accounted for in any such way, nor by postulating any interactions between sex and rations since this was investigated with negative results.

The problem is an interesting and perhaps significant one with possible implications in a wider field of nutrition than hog feeding. It would seem worth while investigating further the nutritive properties of the germ fractions of the cereals and factors which may affect them such as immaturity, freezing and heating. One wonders whether the potency of the germ (in wheat and corn) in nutritional substances may not be correlated with germination tests.

In the meantime the results of these trials indicate that for market hogs barley of good quality can be depended on as the basis of the ration. As the quality of barley declines or as the grain is adulterated with wild oats and weed seeds the feeding value is depressed, largely because of decreased feed consumption. From the standpoint of carcass excellence feed wheat or No. 1 recleaned screenings are perhaps to be preferred to high grade Durum wheat. The latter feed may be expected to produce slightly faster gains in the live hog, but fewer grade A carcasses can be counted on. In so far as carcass grade is concerned, wheat feeding does not compare favourably with any of the feed grades of barley.

CONCLUSIONS

1. As compared to the results obtained by feeding market bacon pigs on rations the basal portion of which was No. 1 feed barley, decrease in weight per bushel of the barley and the presence of the quantities of dockage permitted in the No. 3 feed grade tend to depress rate of gain of the pigs. The effects of these two factors appear to be about equal and they are additive. Average daily gains of 1.74, 1.65, and 1.56 lbs. were obtained from lots fed No. 1 feed barley, No. 1 barley plus dockage, and No. 3 feed barley respectively.

2. Hogs fed wheat as the basal feed made faster gains than those on barley, averaging 1.82, 1.80, and 1.75 lbs. for Durum wheat, No. 1 wheat screenings, and feed wheat respectively.

3. Barley fed hogs yielded more acceptable carcasses as indicated by the proportion receiving the bonus for A grade sides on rail-grading.

4. Carcasses from Durum wheat feeding were penalized for over finish. In type they resembled corn fed carcasses excepting that they showed no tendency to softness.

5. Carcasses from feed wheat and wheat screenings were more satisfactory than those from Durum wheat, but averaged fewer A grades than the barley fed group. Penalties were usually for under finish.

6. Marked differences both in gains and in carcass characteristics were noted between sexes. Males gained faster and had fatter carcasses than females regardless of ration. Females had a larger "eye of lean" in the bacon rasher, while in this group of hogs all males showed an extra streak of lean at the flank end of the rasher not found in any female carcasses. Whether this last is a regularly shown sex difference or whether it is a sex linked hereditary character which may be present or absent

according to breeding is not known. Its presence, however, has been claimed to be desirable from the standpoint of the consumer.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the co-operation of: the Western Canadian Wheat Pools, who supplied all feeds used in the test; the Dominion Department of Agriculture for direct financial assistance and through the Marketing Service for grading all carcasses; and Canada Packers, who provided facilities for special slaughter of the test pigs and absorbed the loss from cutting the carcasses.

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A SOXHLET TYPE APPARATUS FOR THE SIMULTANEOUS EXTRACTION OF A LARGE NUMBER OF SAMPLES[1]

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Where numerous analyses of feeding stuffs are required, the use of a battery of Soxhlet extractors is not only time and space consuming, but

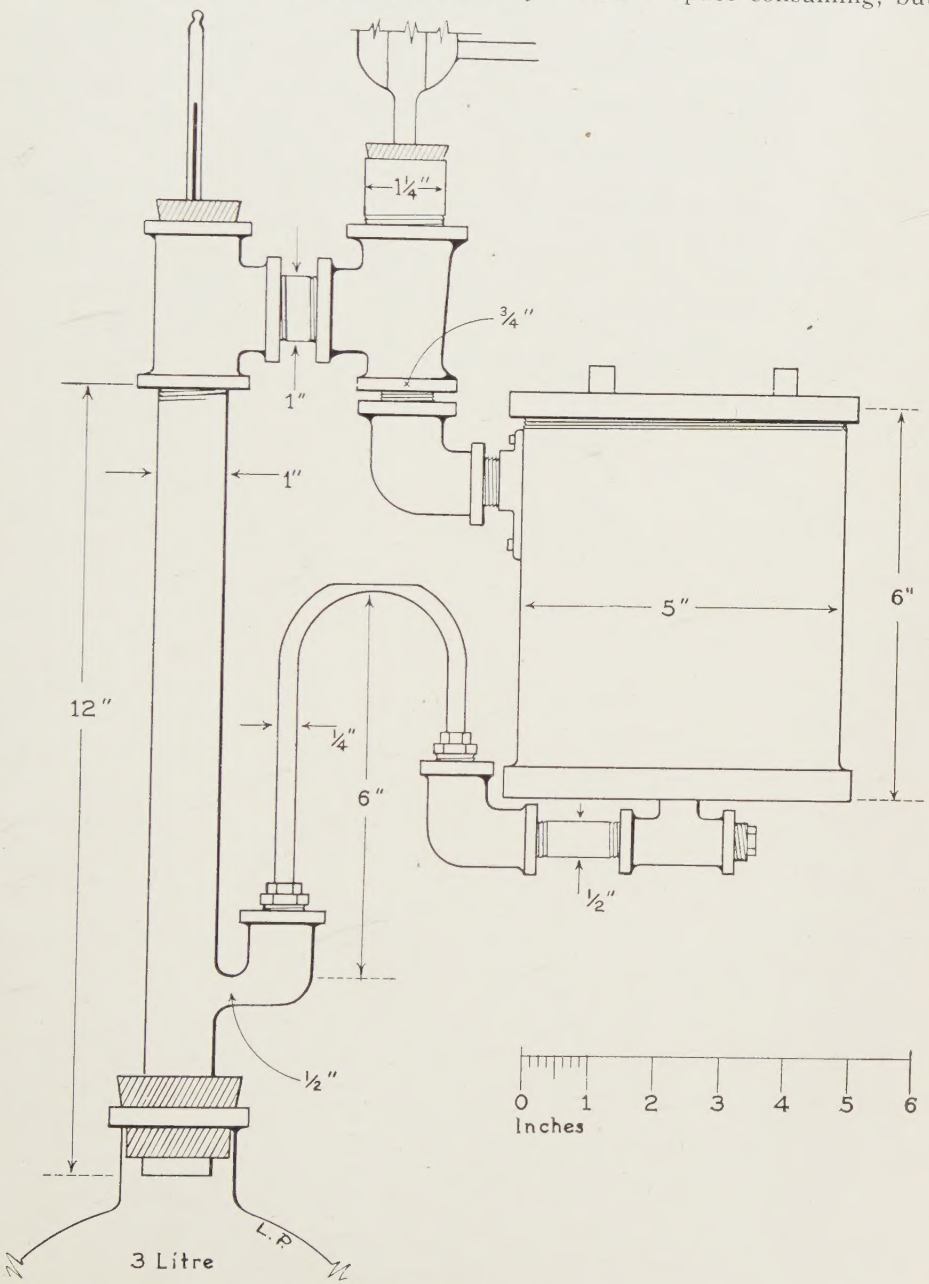

FIGURE 1. Soxhlet apparatus.

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 161.

[2] Associate Professor of Animal Nutrition.

expensive. When "ether extract" is not required or is to be determined by difference, then a single extraction chamber, large enough to hold a number of thimbles should facilitate obtaining the lipid free samples.

The apparatus described here has been used for the past two years with satisfactory results for the ethyl ether extraction of feeding stuffs. It has also been proved useful for the alcohol extraction of bone samples.

The apparatus is essentially a Soxhlet type extractor. Excepting for the glass 3-litre boiling flask and the condenser, it is made entirely from brass or copper. The syphon tube is copper. The extraction chamber is a 6-inch length of 5-inch thin walled brass tubing, to which especially cast brass caps are threaded.

With an efficient condenser and with twelve 5-inch $\times$ 1-inch alundum crucibles in the extraction chamber it is quite possible to make the syphon operate at half-hour intervals, in spite of the large capacity.

It should be noted that the flexible copper tube has been flattened at the top of the curve. Without this, the column of solvent has a tendency to break before the syphoning is complete.

A NEW SERVICE OF BIOLOGICAL ABSTRACTS

To meet the requirements of men engaged in the animal industries, *Biological Abstracts* announces the establishment of a new section, *Section F, Abstracts of Animal Production and Veterinary Science*, beginning January, 1942.

The biological research literature on the breeding, nutrition, husbandry, diseases and pests of the domesticated animals—including poultry, fur-bearing animals and pet stock—is scattered throughout a large number of original research journals in many languages, and its assembly, in the abstract issues and indexes of a single comprehensive abstracting journal will, it is felt, be a great convenience to all who are working in the broad field of animal production.

The new section will consist of ten abstract issues per year. The annual subscription rate will be $5. Subscribers will receive the index to the complete edition of *Biological Abstracts*.

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Inquiries should be addressed to *Biological Abstracts*, University of Pennsylvania, Philadelphia.

JOHN E. FLYNN,
Editor-in-Chief.

ERRATA

The chart appearing on page 219 of the December issue of *Scientific Agriculture* (Vol. 22, No. 4) does not belong with the paper in which it was printed. It belongs with the paper by A. W. Platt entitled "The influence of some environmental factors on the expression of the solid stem character in certain wheat varieties", and appeared in its proper place on page 143 of the November issue (Vol. 22, No. 3).

In the paper "Pasture Studies XXII", by E. W. Crampton and T. L. Purdy in the December issue of *Scientific Agriculture* (Vol. 22, No. 4), reference No. 5 should read *Garrigus, W. P.* and *H. P. Rusk.*